evolution

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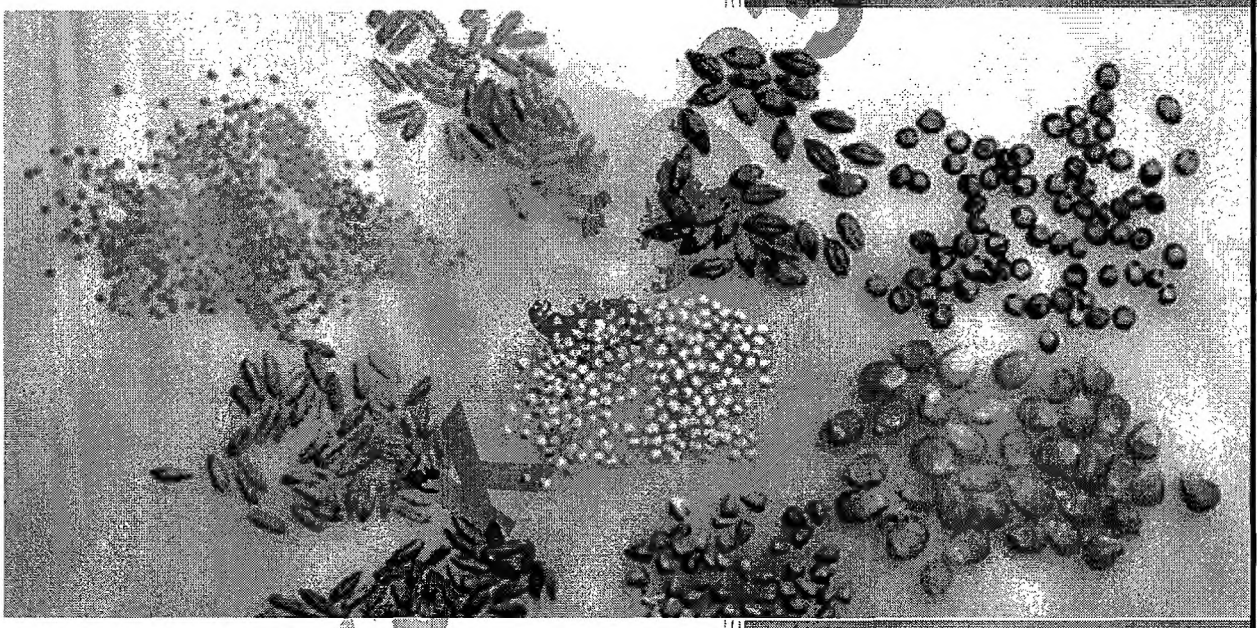
BOATNY: Plant Breeding



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Introduction of Plant Breeding

An art as well as science of improving the genetic makeup of economically important plants to produce desired characters. The sole aim is to improve crop in crop performance paramaters (CPPs), and the list of CPP is never ending.

Methods of Breeding Autogamous species (self-pollinated crops)

Plant breeding methods that are used for genetic improvement of self-pollinated orautogamous species include:

1. Plant Introduction
2. Pure line selection
3. Mass selection
4. Pedigree method
5. Bulk method
6. Single seed descent method
7. Backcross method
8. Heterosis breeding
9. Mutation breeding
10. Polyploidy breeding
11. Distant hybridization
12. Transgenic breeding.

** Four breeding approaches, *viz.*, recurrent selection, disruptive selection, diallele selective mating, and biparental mating are used for population improvement.

Methods or Breeding Allogamous species (cross pollinated crops)

Breeding methods that are used for genetic improvement of cross pollinated orallogamous species include

1. Plant introduction
2. Mass and progeny selection
3. Backcross method
4. Heterosis breeding
5. Synthetic breeding
6. Composite breeding
7. Polyploidy breeding
8. Distant hybridization
9. Transgenic breeding

** Mutation breeding is rarely used in allogamous species. Three breeding approaches *viz.*, recurrent selection, disruptive mating and biparental meting are used for population improvement.

Methods of Breeding Asexually Propagated Species

Important breeding methods applicable to asexually propagated species are

1. Plant Introduction
2. Clonal selection
3. Mass selection (seldom done)
4. Heterosis breeding
5. Mutation breeding
6. Polyploidy breeding
7. Distant hybridization
8. Transgenic breeding.

Brief account of breeding methods

Plant introduction is applicable to all three groups of crop plants, *viz.*, self-pollinated, cross pollinated and asexually propagated species. It is an oldest and rapid method of crop improvement. The introduced material may be used in three ways *viz.*,

1. Directly as a variety
2. As a variety after selection
3. As a parent in the hybridization for development of variety or hybrid

Mass selection is common in cross pollinated species and rare in self-pollinated and asexually propagates species. In self-pollinated crops, a mass selected variety is a mixture of several pure lines. Thus it is a homozygous but heterogeneous population. In cross pollinated species, a mass selected variety is a mixture of several hetero and homozygotes. Thus, it is a heterozygous and heterogeneous population.

Progeny selection is used in cross pollinated species. A variety developed by this method is heterozygous and heterogeneous population because it consists of several hetero and homozygotes.

Pure line selection is applicable to self-pollinated species. It is also used sometimes in cross pollinated species for development of inbred lines. A single best pure line is released as a variety. Thus a pure line variety is homozygous and homogeneous population.

Pedigree method is applicable to both self and cross pollinated species. In self-pollinated crops progeny of a single best homozygote is released as a variety. Thus a variety developed by this method has a homozygous and homogeneous population. In cross-pollinated species, it is used for developed of inbred lines.

Bulk and single seed descent methods are used in self-pollinated species. Progeny of a single best homozygote is released as a variety by these methods. Thus, varieties developed by these methods are homozygous and homogeneous.

Backcross method is applicable in all three groups of crop species. This method is used for transfer of oligogenic characters from a donor source to a well-adapted variety. This method is also used for development of **multiline, Isogenic lines** and transfer of male sterility. This method is more effective in transferring oligogenic characters than polygenic traits. The end product of backcross method is similar to parent variety expect for the character which has to be transferred from the donor source. **Multiline varieties** are developed in self-pollinated species. They are mixture of several Isogenic lines, closely related lines or unrelated lines. Thus a multiline variety is ahomozygous but heterogeneous population. **Clonal selection** is used in asexually propagated species. In this method progeny of a single best clone is released as a variety. Such variety has heterozygous but homogeneous population.

Heterosis breeding is used in/all the three groups. However, it is common in cross-pollinated and asexually propagated species and rare in self-pollinated species. A hybrid variety has homogeneous but heterozygous population. Synthetic and composite varieties are developed in cross pollinated species. Such varieties consist of several homozygotes and heterozygotes and thus constitute a heterogeneous population.

Mutation breeding is common in self-pollinated and asexually propagated species and rare in cross pollinated species. A mutant variety differs from parent variety in one or few characters. A mutant differs from a segregant in two main ways. Firstly, the frequency of segregants is very high and that of mutant is extremely low (0.1%). Secondly, mutant differs from parent variety in one or few characters, where as a segregant differs from parent material in several characters.

Polyploidy breeding is common in asexually propagated species and rare in self and cross pollinated species. A polyploidy variety differs from parent variety in chromosome numbers and exhibit gigant morphological characters.

Distant hybridization is used in all the three types of crop species. However, this method is used for transferring some desirable genes from wild species to the cultivated ones. Generally, backcross method is used for transfer of oligogenic characters and pedigree method for transfer of polygenic characters.

Transgenic breeding is applicable to all three types of crop species. This method is used to solve specific problems which cannot be solved by conventional breeding techniques. This method will serve as a tool and cannot be used as a substitute for conventional breeding methods.

Recurrent selection is common in cross pollinated species and rare in other two groups. It is used for accumulating favourable genes in a population *i.e.,* for population improvement. Other approaches which are used for population improvement include *disruptive mating, diallel selective mating (DSM) and biparental mating*. DSM is used in self-pollinated species and other two techniques can be used both in self and cross pollinated species.

Plant introduction

Plant introduction consists of taking a genotype or a group of genotypes of plants into new environments where they were not being grown before. Introduction may involve new varieties of a crop already grown in the area, wild relatives of the crop species or a totally new crop species. Mostly materials are introduced from other countries or continents. But movement of crop varieties from one environment into another within a country is also introduction. Thanks to N. I Vavilov, who after a great deal of study found that although many crops are grown all over the world, they actually originated at one or few place. He determined centres of origin of domesticated crops (eight of them) and believed that the varieties grown in the centres of origin usually contained a large number of dominant genes with recessive gene never showing up due to nearly complete adaptation of the variety to its native site, but the same crop in different environmental conditions showed new phenotypic characters due to changes in environmental condition leading to mutation in recessive gene making then important in new isolated regions. These centres are geographical areas where the species had originally established either as domesticated from wild or as wild only.

His important finding can be concluded as:

1. 85% of 640 crop species are in old world.
2. There are small areas in tropics and subtropics which are endowed with greatest wealth of forms, gene or diversity centres and from these centres the species have migrated in different direction in the course of which they have shown adaptive changes and these accumulation of variation led to formation of cultivars
3. Primary crops have multiple origins viz. Wheat, Rice, potato, Soybean, Flax, Cotton.

4. When primary crops along with forage crop or weed were taken from one place to another very distant place beyond many climatic zones, the forage became the primary and primary became weed or forage due to radical changes in the environmental conditions, thus gradually primary crops and associated crops are said to have multiple origins as they are found with many different civilization as part of their cultural and ethnic diet.
5. Genetic diversity within a species at its centre of origin may or may not be very high. There are cases when higher genetic diversity is more in secondary centres e.g. maize and tomato
6. He proposed primary and secondary centre of origin.
7. **Primary centre** are those which are characterized by dominant gene expression with maximum number of wild relative present nearby. It was their cultivation since ancient times that has led to diversity in that species, if any so basically longer periods of perpetuation of a number of crop species is important here.
8. **Secondary centre** or accumulation gene centres formed due to human migration patterns over long distances across physical barriers. Secondary centres are characterized by recessive allele expression that is neo types are common here. There are no wild relatives even in 1000 km radius. It was natural forces that led to the diversity. So, ecological diversity, farming practice, human migration patterns towards different races of a crop are important here.

Examples of recent introduction were tobacco, Potato, maize in Asia, Ridley variety of wheat from Australia to India (resistant to brown and black rust, and to loose smut with good yield and grain quality). Sometimes a suitable or desired line or strain of a crop has to be introduced e.g. a **mung (*Phaseolus aureus*)** variety from **China** was introduced in India, but the variety gave poor yield and dull coloured seeds but during ongoing selection one single plant was found having large and bright colour seeds, **Shining mung no. 1 variety** of Punjab is

the descendant of this single plant. Similarly, in many cases introduced varieties have been used as parents in crosses and their gene utilised in future progenies e.g. four resistant varieties of linseed were brought to India from Australia and with suitable crosses rust resistant genes were transferred to the Indian rust susceptible varieties of linseed.

What is required is **planned introduction** and for this it is essential to have a knowledge and collection of diverse genotypes of a species that can be used as a source material for desirable gene or characters and this germplasm collection includes local as well as exotic strains of the crop plants and its related species, which is used to genetically improve the species.

Based on the area of introduction, plant introduction can be of two types: **Intra-country** (Grapes in Haryana, Rice in Punjab and wheat in Bengal and rest of the non-wheat growing areas of North East) and **Inter-country** introduction (Major type viz. Maize, Potato).

Based on the activity with these plants, they can be two types: Primary and Secondary

1. **Primary introduction**: When the introduced variety is well adapted to new environment and do not require to be manipulated and is straightforwardly released for commercial cultivation. This kind of introduction is less common in countries which have their own well developed crop improvement programme. For example introduction of semi dwarf variety of wheat varieties Sonara 64, Lerma Rojo, Semi dwarf rice varieties IR8, IR28, Taichung native 1 etc.
2. **Secondary introduction**: The original genotype of the variety to be introduced is altered and subjected to selection to isolate the better performing progenies from the rest or better still the superior progenies are further hybridized with local varieties to transfer one or few characters from this variety to the local ones. E.g. Kalyan Sona and Sonalika wheat varieties developed from Mexican wheat genotypes.

Plant introduction for self and cross pollinated species serves the same purpose of increasing the options to improve a crop species or to simply broaden up the genetic base of a cultivar.

Application of Plant Introduction

1. To increase food options, by providing new crop species or varieties thus increase food security, e.g. Potato, maize etc.
2. To directly introduce better yielding commercial hybrids or CVs for profitable production of crop by the farmers thus improving farmer's condition.
3. Utilisation of introduced close relatives of an existing species into the crop improvement breeding programmes. E.g. Pusa ruby tomato was derived from a cross between a local variety (merruty) with Sioux from USA
4. Plant introductions helps in disease and pest management as sometimes a crop introduced into a new areas helps in the escaping from its natural predators and also in a new environment it has no natural enemies if that variety or species has not been growing there previously, e.g. Coffee was introduced in South America from Africa to protect it from leaf rust and Hevea was brought to Malaya from South America to protect it from a bacterial leaf disease.

Plant Introduction Agencies in India

A centralized plant introduction agency was initiated in 1946 at the Indian Agricultural Research Institute (IARI), New Delhi. The agency began as a plant introduction scheme in the Division of Botany and was funded by ICAR. In 1956, during the second five year plan, the scheme was expanded as the Plant Introduction and Exploration Organisation. Subsequently in 1961, it was made an independent division in IARI, the Division of Plant Introduction. The division was re-organized as National Bureau of Plant Genetic Resources (NBPGR) in 1976. The nature of activities and the functions of the bureau have remained the

same, but the scope and scale of its activities have increased considerably. The bureau is responsible for the introduction and maintenance of germplasm of agricultural and horticultural plants. In addition to the National Bureau of Plant Genetic Resources, there are some other agencies concerned with plant introduction. Forest Research Institute, Dehradun, has a plant introduction organization which looks after the introduction, maintenance and testing of germplasm of forest trees. The Botanical Survey of India was established in 1890; it was responsible for the introduction, testing and maintenance of plant materials of botanical and medicinal interest. But at present, introduction and improvement of medicinal plants is being looked after by NBPGR. The Central Research Institute for various crops, e.g., tea, coffee, sugarcane, potato, Tobacco, rice etc., introduce, test and maintain plant materials of their interest. But their activities are coordinated by the NBPGR, which has the ultimate responsibility for introduction activities. Plant material may also be introduced by individual scientists, universities and other research organizations. But all the introductions in India must be routed through the NBPGR, New Delhi.

The National Bureau of Plant Genetic Resources has its headquarters at IARI, New Delhi. It has four substations for the testing of introduced plant materials. These substations represent the various climatic zones of India which are most suitable to respective crop material, they are as follows:

1. **Simla, Himachal Pradesh**. It is situated in Himachal Pradesh and represents the temperate zone; approximately 2,300 m above sea level.
2. **Jodhpur, Rajasthan**. It represents the arid zone.
3. **Kanya Kumari, Tamil Nadu**. It represents the tropical zone
4. **Akola, Maharashtra**. It represents the mixed climatic zone. It was recently shifted from Amravati.

In addition, a new substation has recently been established at Shillong for collection of germplasm from North-east India. This part of the country has a large genetic variability for several crop species, e.g., rice, citrus, Maize etc. The

bureau functions as the central agency for the export and introduction of germplasm of economic importance. The bureau is assisted in its activities by the various Central Research Institutes of ICAR.

The activities of the bureau are summarized below.

1. It introduces the required germplasm from its counterparts or other agencies in other countries.
2. It arranges explorations inside and outside the country to collect valuable germplasm.
3. It is responsible for the inspection and quarantine of all the introduced plant materials.
4. Testing, multiplication and maintenance of germplasm obtained through various sources. This may be done by the bureau itself at one of its substations or by one of the concerned Central Institutes of ICAR.
5. To supply, on request, germplasm to various scientists or institutions. The germplasm may be supplied ex-stock or may be procured from outside in case it is not available in the country.
6. Maintenance of records of plant name, variety name, propagating material, special characteristics, source, date and other relevant information about the materials received.
7. To supply germplasm to its counterparts or other agencies in other countries.
8. To publish its exchange and collection lists. An Introduction News Letter containing such lists is being published by the Food and Agriculture Organisation (FAO) since 1957 at irregular intervals. NBPGR has also published some lists, and is in the process of publishing some other catalogues.
9. To set up natural gene sanctuaries of plants where genetic resources are endangered.
10. Improvement of certain plants like medicinal and aromatic plants.

Procedure of Plant Introduction

Introduction consists of the following steps: Procurement, quarantine, cataloguing, evaluation, multiplication and distribution.

1. **Procurement**: Any individual or institution can introduce germplasm in India. But all the introductions must be routed through the NBPGR, New Delhi. There are two routes for plant introduction. In the first route the individual or the institution makes a direct request to an individual or institution abroad, which has the desired germplasm, to send it through the NBPGR, New Delhi. In second procedure the individual or institute submits his germplasm requirements to the NBPGR with a request for their import.
2. **Quarantine:** Quarantine means to keep materials in isolation to prevent the spread of diseases to a new area where there has been no report of that disease or pathogen or pathotype whatsoever be the case may be. All the introduced plant propagules are thoroughly inspected for contamination with weeds, diseases and insect pests. Materials that are suspected to be contaminated are fumigated or are given other treatments to get rid of the contamination. If necessary, the materials are grown in isolation for observation of diseases, insect pests and weeds. The entire process is known as quarantine and the rules prescribing them are known as quarantine rules.
3. **Cataloguing:** When an introduction is received, it is given an entry number. Further, information regarding name of the species, variety, place of origin, adaptation and its various characteristics are recorded. The plant materials are classified into three groups.
   a. Exotic collections are given the prefix 'EC'
   b. Indigenous collections are designated as 'IC' and
   c. Indigenous wild collections are marked as 'IW'.
4. **Evaluation:** To assess the potential of new introductions, their performance is evaluated at different substations of the Bureau. In case of

those crops for which Central Research Institutes are functioning, e.g., rice, sugarcane, potato, Tobacco etc., the introduced materials are evaluated and maintained by these institutes. The resistance to diseases and pests is evaluated under environments favouring heavy attacks by them.

5. **Multiplication and Distribution**: Promising introductions or selections from the introductions may be increased and released as varieties after the necessary trials. most of the introductions, however, are characterized for desirable traits and are maintained for future use. Such materials are used in crossing programmes and are readily supplied by the bureauon request.

Purpose of Plant Introduction

The main purpose of plant introduction is to improve the plant wealth of the country.

The chief objectives of plant introduction may be grouped as follows:

1. **To obtain an entirely new crop plant**: e.g., Maize, potato, tomato, Tobacco, etc., are introductions. Some recently introduced crops are Soybean, Cauliflower and Cabbage, Broccoli, Brussels Sprout, oil palm etc.
2. **To serve as new varieties which are directly released as superior commercial varieties**: e.g. Mexican semi dwarf wheat varieties Sonora 64 and Lerma Rojo, semidrawf rice varieties TN 1, IR-8 and IR-36 are more recent examples of this type.
3. **To be used in crop improvement by hybridization with existing cultivars**: e.g. Pusa Ruby tomato was derived from across between Meeruty and Sioux, an introduction from U.S.A. Another example is all the semi dwarf wheat varieties are derived from crosses with Mexican semi-dwarf wheat. Semi dwarf rice varieties possess the dwarfing gene from Dee-geo-woo-gen through either TN1 or IR8. Thus almost all these semi-dwarf wheat and rice varieties have been developed from crosses

involving introductions. All the sugarcane varieties have been derived from the introduced noble canes. Pusa Early Dwarf Tomato derived from the cross Meeruti x Red Cloud; Pusa Kesar carrot, Pusa Kanchan turnip etc.

4. **To save the crop from diseases and pests:** e.g. Coffee was introduced in South America from Africa to prevent losses from leaf rust. *Hevea* (Rubber) was brought to Malaya from South America to protect it from a leaf disease.
5. **Collections of plants have been used for studies on biosystematics, evolution and origin of plant species.** N. I. Vavilov developed the concept of centres of origin and that of homologous series in variation from the study of a vast collection of plant types.
6. **For Aesthetic Value.** Ornamentals, shrubs and lawn grasses are introduced to satisfy the finer sensibilities of man. These plants are used for decoration and are of great value in social life.
7. **Varieties Selected from Introductions:** many varieties have been developed through selection from introductions. Two varieties of wheat, Kalyan Sona and Sonalika, were selected from introductions from CIMMYT, Mexico.

Merits of Plant Introduction

1. It provides entirely new crop plants.
2. It provides superior varieties either directly or after selection & hybridization.
3. Introduction and exploration are the only feasible means of collecting germplasm and to protect variability from genetic erosion.
4. 4. It is very quick & economical method of crop improvement, particularly when the introductions are released as varieties either directly or after a simple selection.

5. Plants may be introduced in new disease free areas to protect them from damage, e.g., coffee and rubber.

Demerits of Plant introduction as witnessed in recent past

1. Many introduced plants bring with them, a number of weed species, diseases and pests. This happens due to similarity in seed size, shape and colour with the seeds of plant to be introduced, e.g. *Argemone Mexicana* and *Parthenium* were brought along with cereal crops etc.
2. Sometimes these alien species which are introduced become invasive species or in other words become weeds e.g. *Lantana camara* bought in for its ornamental value.
3. Threat to ecological balance due to encroachment of habitats with aliens.
4. No guarantee of whether the introduced plant will become a success and cater to the need of growing population.

Selection as method of plant breeding

Selection is one of the oldest procedures used for crop improvement and during this process individual plants or groups of plants are sorted out from mixed population. It is basic to any crop improvement. Isolation of desirable plant types from the population is known as selection. It is one of the two fundamental steps of any breeding programme viz., 1. Creation of variation and 2. Selection.

There are two agencies involved in carrying out selection: one is Nature itself (Natural selection) and the other is by man that is artificial selection. Though both may complement each other in some cases, they are mostly opposite in direction since their aims are different under the two conditions (nature and domestication).

The effectiveness of selection primarily depends upon the degree to which phenotype reflects the genotype. Before domestication, crop species were subjected to natural selection. The basis for natural selection was adaptation to the prevailing environment.

After domestication man has knowingly or unknowingly practiced some selection. Thus crop species under domestication were exposed to both natural and artificial selection i.e. selection by man. For a long period, natural selection played an important role than selection by man. But in modern plant breeding methods natural selection is of little importance and artificial selection plays an important role.

Selection as a tool can happen only when we have variation, as without variation selection is meaningless. It acts as a sieve in favour of well adapted more fit strains and varieties. It applies to both self and cross pollinated species. To understand the potential of selection, it is enough to say that present day plants

are quite different from the earliest ones because they have evolved as a result of variation and selection of most suited variants.

Basic Principles of Selection

Notwithstanding the highly complex genetic situation imposed by linkage and epistasis, there are just three basic principles of selection as given by Walker in 1969. These are:

1. **Selection operates on existing variability**: The main function of the selection exercise is to discriminate between individuals. This is possible only when sufficient variation is present in the material subjected to selection pressure. Thus, selection acts on the existing variation, it cannot create new variation.
2. **Selection acts only through heritable differences**: only the selected individuals are permitted to contribute to the next generation/progenies. Therefore, should there be greater influence of non-heritable agencies on the individuals selected; the parent-progeny correlation will be greatly vitiated. Hence the variation among individuals to be selected must be genetic in nature, since it is the genetic variation that tends to close the gap between phenotype and genotype. Environmental variability cannot be of any use under selection.
3. **Selection works because some individuals are favoured in reproduction at the expense of others**: As a consequence of its past evolutionary history and breeding structure, a population or a crop consists of highly genetically variable individuals with regards to such diverse phenomena as differential viability, differential maturity, differences in mating tendencies, fecundity, and duration of reproductive capacity. Hence some individuals tend to become superior to others for some or other traits desirable under domestication. These superior individuals are retained for reproduction while others discarded under selection.

Recurrent selection

A cyclical improvement technique aimed at gradually concentrating desirable alleles in a population. These are population improvement breeding programmes. These require extensive crossing (and if, the crop is SPC then we need male sterile lines). These are effective in improving QTLs. They require controlled environment. Success of a RS programme rest on the genetic nature of base population, higher the breeding value is the better will be the outcome. RS is superior to classic breeding when linkage disequilibrium exist.

Purpose

It is to improve the performance of a population with respect to one or more traits such that the new population is superior to the original population in mean performance and in the performance of the best individuals in it. Most desirable outcome of recurrent selection is that the improved population is produced without the reduction in genetic variability which is a common concern in conventional breeding.

Key factors

1. Parents should be high performance regarding the TOI and should not be closely related.
2. Inclusion of as many parents as possible in initial crossing to increase genetic diversity.
3. As crossing provide opportunity of recombination of Gene to increase genetic diversity of the population, so more rounds of mating will increase the opportunity for recombination, although this will increase the breeding programme duration.

A RS cycle has three main phases:

1. Individuals families are created for evaluation. Parents are crossed in all possible combinations.
2. The plants/families are evaluated and a new set of parents selected.
3. Selected parents are inter-mated to produce for the next cycle of selection.

This cycle is repeated several times (3-5). First cycle (C_0) is base population and subsequent cycles are C_1, C_2, C_3...C_n.

Types of recurrent selection: based on how plants with desired traits are identified

1. **Simple RS**: similar to mass selection with 1 or 2 years/cycle. No tester is used. Selection is based on phenotypic scores also called phenotypic recurrent selection.
2. **RS for GCA**: here a wide genetic based cultivar is used as a tester. The test cross performance is evaluated in replicated trials prior to selection. Basically half sib progeny test is done.
3. **RS for SCA**: This scheme uses an inbred line (narrow genetic base) for a tester. The test cross performance is evaluated in replicated trials prior to selection.
4. **Reciprocal RS**: This scheme is capable of both GCA and SCA entailing two heterozygous populations each serving as a tester for the other. Both half and full sib evaluation is done here.

Advantages

1. Opportunity to break linkage block exists because of repeated inter-crossing.
2. It is applicable to both auto-gamous grasses (Barley and Oats) as well as legumes (Peanuts and Soybean).

Disadvantages

1. Extensive crossing is required which is very challenging in autogamous species.
2. Sufficient seed may not be available after inter-crossing.
3. More intermating may prolong the duration of breeding programme.
4. There is also the possibility of breaking desirable linkages.

Hybridisation

It is the dominant breeding approach in 20th century both in self and cross pollinated crops. It essentially means crossing two parents with desirable traits to obtain a progeny which has acquired the best of both the parents. The main objective thus is to combine desirable genes found in 2 or more varieties and to produce superior progenies with respect to parental types.

The chief objective of hybridization is to create genetic variation. When two genotypically different plants are crossed, the genes from both the parents are brought together in F_1. Segregation and recombination produce many new gene combinations in F_2 and in the later generations, i.e. the segregating generations. The degree of variation produced in the segregating generations would, therefore, depend on the number of heterozygous genes in the F_1. This still, in turn, depends upon the number of the genes for which the two parents differ. If the two parents are closely related, they are likely to differ for a few genes only. But if they are not related, or are distantly related, they may differ for several, even a few hundred, genes. The aim of hybridization may be the transfer of one or few qualitative characters, the improvement in one or more quantitative characters.

Hybrid Varieties

In most self-pollinated crops, F_1 is more vigorous and higher yielding than the parents. Wherever it is commercially feasible, F_1 may be used directly as a variety. In such cases, it is important that the two parents should produce an outstanding F_1.

Depending on the objective and case, breeding through hybridization can be of following types:

Combination Breeding: The main aim of combination breeding is the transfer of one or more characters into a single variety from other varieties. These

characters may be governed by oligogenes (few genes) or polygenes (many genes). The intensity of the character in the new variety is either comparable to or, more generally, lower that in the parent variety from which it was transferred. In this approach, increase in the yield of a variety is obtained by correcting the weaknesses in the yield contributing traits, e.g., tiller number, grains per spike, test weight is that for disease resistance. The backcross method of breeding was designed for combination breeding, and often pedigree method also fulfils the same purpose. In combination breeding, the genetic divergence between parents is not the major consideration. What is important is that one of the parents must have in a sufficient intensity the character(s) under transfer, while the other parent is generally a popular variety.

Transgressive Breeding: Transgressive breeding aims at improving yield or its contributing characters through transgressive segregation. Transgressive segregation is the production of plants in an F_2 generation that are superior to both the parents for one or more characters. Such plants are produced by an accumulation of plus or favourable genes from both the parents as they must combine well with each other, and should preferably be genetically diverse, i.e., quite different. This way, each parent is expected to contribute different plus genes which when brought together by recombination give rise transgressive segregant. As a result, the intensity of character in the transgressive segregant, i.e., the new variety, is greater than that in either of the parents. The pedigree method of breeding and its modifications, particularly the population approach, are designed for the production of transgressive segregants.

Types of CVs to be released to producers as a factor governing the choice of breeding method to be used by breeder

Before initiating any breeding program, the breeder has to decide which breeding method he wants to follow and why. Among many other factors, one of the most important factor is which type of cultivar he wants to release at the end of the breeding program and based on the type of CV, the breeding methods are chosen.

These cultivar are derived from four basic populations namely, inbred pure lines, open pollinated populations, hybrids and clones. Types of CVs are:

1. Pure Line Cultivars:

a. developed for highly self-pollinated sp.
b. CVs are homozygous and homogenous, often used as parents in production of new kinds of cultivars

2. Open pollinated Cultivars:

a. developed for species that are naturally cross pollinated
b. CVs are genetically heterogenous and heterozygous
c. two basic types are developed one is developed by improving the general population by recurrent selection or bulking and other type is derived from planned mating involving selected genotypes called synthetic cultivars
d. these have broad genetic base.

3. Hybrid cultivars:

a. produced by crossing two inbred lines that have been evaluated to produce sup. Progeny with vigour over and above the inbred parents
b. exploits Heterosis in production of superior progenies
c. mainly for cross pollinated species, less important for self-pollinated species as Heterosis has no significant increase in vigour to offer there.
d. CVs are homogenous but heterozygous.

4. Clonal CVs

a. CVs from vegetative propagating plant parts such as stem and root, so basically they are clones and thus highly homogenous but genetically highly heterozygous as they are obtained either through a hybridisation program or are naturally cross pollinated as these are the only two ways to fix Heterosis and or variations in such vegetatively propagating plants.

5. Apomictic CVs

a. Apomixis being the phenomenon of producing seed without gametic union means that the seeds are essentially clones so highly homogenous but are heterozygous as that is the only way apomictic plants incorporate variations in them, thus they are generally cross pollinated.
b. Most perennial forage grasses are apomictic and thus there Apomixis is exploited to develop apomictic CVs for use in forage.
c. apomictic CVs have the same benefits of clonally propagated CVs apart from the fact that here seeds play the role of vegetative parts such as cuttings etc. which is unusual.

6. Multiline

a. developed for self-pollinated species
b. these CVs consist of a mixture of specially developed genotypes called isolines or near isogenic lines, differing in single gene set and are potentially used in disease resistance, environmental stress resistance etc.
c. developed by backcrossing F_1 with recurrent parent lacking the GOI.

Methods of breeding in Self Pollinated Crops

Mass selection

It is the earliest method of selection. Man has always practiced mass selection consciously or unconsciously from the time of domestication. In its most basic form mass selection consists of selecting individuals on the basis of phenotypic superiority and mixing the seeds for using as planting material for next season.

Procedure for evolving variety by mass selection

First year: Large numbers of phenotypically similar plants having desirable characters are selected. The number may vary from few hundred to thousand. The seeds from the selected plants are composited to raise the next generation.

Second year: composited seed planted in a preliminary field trial along with standard checks. The variety from which the selection was made should also be included as check. Phenotypic characteristics of the variety are critically examined and evaluated.

Third to sixth year: The variety is evaluated in coordinated yield trials at several locations. It is evaluated in an initial evaluation trial (IET) for one year. If found superior it is promoted to main yield trials for 2 or 3 years.

Seventh year: if the variety is proved superior in main yield trials it is multiplied and released after giving a suitable name.

Modification of mass selection

Mass selection is used for improving a local variety. Large number of plants are selected (I year) and individual plant progenies are raised (II year). Inferior, segregating progenies are removed. Uniform, superior rows are selected and the seed is bulked. Preliminary yield trials are conducted in third year. Fourth to

seventh year multi location tests are conducted and seed is multiplied in eight year and distributed in ninth year. Many other modifications also are followed depending on the availability of time and purpose for which itis used.

Merits of Mass selection

1. Can be practiced both in self and cross pollinated crops
2. The varieties developed through mass selection are more widely adopted than pure lines.
3. It retains considerable variability and hence further improvement is possible in future by selection
4. Helps in preservation of land races
5. Useful for purification of pure line varieties
6. Improvement of characters governed by few genes with high heritability is possible.
7. Less time consuming and less expensive.

Demerits of mass selection

1. Varieties are not uniform
2. Since no progeny test is done, the genotype of the selected plant is unknown.
3. Since selection is based on phenotype and there is no control over pollination the improvement brought about is not permanent. Hence, the process of mass selection has to be repeated now and then.
4. Characters which are governed by large number of genes with low heritability cannot be improved.
5. It cannot create any new genotype but utilizes existing genetic variability.
6. Seed certification problem occurs in mass selected variety because variety is more difficult to identify.

Achievements of mass selection

Mass selection must have been used by pre-historic man to develop present day cultivated cross from their wild parents. It was also used extensively before pure line selection came into existence. Some of the recent achievements are as follows.

1. **Cotton :** Dharwad American Cotton
2. **Groundnut** : TMV-1 & TMV-2
3. **Bajra:** pusamoti, Baja puri, Jamnagar giant, AF3
4. **Sorghum** : R.S. 1
5. **Rice:** SLO 13, MTU-15
6. **Potato**: K122
7. **Maize**: T41, Jaunpuri

A modification of mass selection is progeny selection where progeny is tested although phenotypically. In essence, two cycles of regular mass selection comprise one of progeny selection.

Pure line selection

Developed by Johannsen in 1903, pureline selection aka individual plant selection has been the most commonly used method of improvement of self pollinated crops. Almost all the present day varieties of self pollinated crops are pure lines. Just like any other selection method, it utilizes the variability already present in the population. Pure line selection has several applications in improvement of self pollinated crops. It is usedto improve,

1. Local varieties
2. Old pure line varieties and
3. Introduced varieties

Pure line theory

1. Lines that are genetically different may be successfully isolated from within a population of mixed genetic type.
2. Any variation that occurs within a pure line is usually not heritable but due to environmental factors.

Main features of pure line variety:

1. All the genotypes of pure line are homogenous
2. Variation if present is non-heritable and due to environmental factor
3. The pure line varieties have narrow adaptability due to narrow genetic base and so high mutation rates occur in such genotypes.
4. Pure lines become genetically variable with time.
5. Most of the success of this method depended upon the existence of genetically variable land varieties that were waiting to be exploited
6. The method is now more popular for less important species that have not yet been heavily selected.

General procedure for evolving a variety by pure line selection

The pure line selection has three steps:

1. Selection of individual plants from a local variety or some other mixed population.

2. Visual evaluation of individual plant progenies,

3. Yield trials

1. Selection

Istyear: A large number of plants (200-3000) which are superior to the rest are selected from a local variety or mixed population and harvested separately (in some cases individual heads or stems may be selected). It is advisable to select

for easily observable characters such as flowering, maturity, disease resistance, plant height etc.

2. Evaluation

2nd year: Progenies of individual plants selected in 1st year are grown separately with proper spacing (plant to row or head to row). The progenies are evaluated by taking elaborate data on visual characters such as plant height, duration, grain type, ear characters besides yield. The number of progenies should be reduced as much as possible. Disease epiphytotics (epidemics) may be created to test the progenies for disease resistance, poor, weak, diseased, insect attacked and segregating progenies are rejected. The superior progenies are harvested separately. If necessary the process may be repeated for one or more years.

3. Yield trials

3rd year: The selected progenies, now called as cultures are grown in replicated trial for critical evaluation of yield etc. The best local variety is used as a check and should be grown at regular intervals, after every 15 or 20 cultures for comparison. This is known as preliminary yield trial. Superior cultures based on observable characters and yields are selected. The number is drastically reduced.

4th and 5th years: The superior cultures are tested against the local checks in yield trials. Observations are recorded on many characters like diseases resistance, days to flower, and days to maturity, height of the plant ear characters, test weight and yield. The data is subjected to statistical analysis to identify really superior cultures. If necessary the trials may be extended for one more year or season. Inferior culture are rejected and a few (4-5) promising cultures are selected.

6th, 7th, and 8th year: The promising cultures selected are evaluated at several locations (minimum 5) along with strains or cultures of other breeders and local checks. One or two promising cultures are selected.

9th year: The best progeny identified earlier is multiplied, named and released as a variety for official release of any variety (approval from the variety releasing committee of the state or central is necessary).

Advantages of pure line selection

1. Rapid breeding method and inexpensive too. Base population can be a landrace with the size of population depending on objectives.
2. Pure lines achieve the maximum possible improvement over the original variety.
3. The variety is extremely uniform and more attractive than mass selection.
4. Extremely uniform since all the plants in the variety will have the same genotype. Thus the variety has a great eye appeal and thus become attractive and liked by the farmers and consumers. CVs have importance mainly in mechanized production or food processing industries due to their uniform size and shape.
5. Pure lines are stable for many years.
6. Due to its extreme uniformity the variety can be easily identified in see decertification programmes.
7. A variation of pure line selection method that dates back centuries is identification and selection of single chance variants or better called "sports" of original variety. All the varieties that differ from the original in characters such as colour, lack of thorns, dwarfness, and disease resistance have originated in this fashion.
8. It is applicable to improving traits of low heritability because selection is based on progeny performance. Mass selection may include some inferior pure lines but in pure lines selection, only the best pure lines are selected for maximum genetic advance.

Disadvantages of pure line selection

1. New genotypes are not created by pure line selection

2. Improvement is limited to the isolation of the best genotype present in population. No more improvement is possible after isolation of the best available genotype in the population.
3. Selection of pure line variety requires great skill and familiarity with the crop.
4. Difficult to detect small differences that exist between cultures
5. The breeder has to devote more time,
6. Pure lines have limited adaptability hence can be recommended for cultivation in limited area only. Narrow adaptability is due to narrow genetic base and so high mutation rates occur in such genotypes. Also, narrow genetic base makes the CV susceptible to devastation from adverse environmental factors. In other words this method promotes genetic erosion because most superior pure lines are identified and multiplied to the exclusion of other genetic variants.
7. Progeny rowing takes up more resources and funds.
8. Most of the success of this method depended upon the existence of genetically variable land varieties that were waiting to be exploited. Thus this method is now more popular for less important species that have not yet been heavily selected.
9. Variation if present in a pure line variety is non-heritable and due to environmental factors.

Achievements of Pure line selection

Several varieties developed by pure line selection which have been released are:

1. **Rice:** Mtu-1, Mtu-3, Mtu-7, Bcp-1, Adt-1, 3, 5, and 10, Shyama (this one is improvement of an old existing pure line variety)
2. **Sorghum**: G 1 & 2, M 1 & 2, OO 1, 4 & 5,
3. **Groundnut** : TMV 3, 4, 7, 8 and Kadiri 71-1
4. **Red gram :** TM-1, ST-1
5. **Chillies** : G1 & G2

6. **Ragi :** AKP 1 to 7
7. **Wheat:** NP-4, NP-52, Kalyan Sona (this one is through pure line selection in an introduced variety from Mexico)
8. **Linseed**: NP-11, NP-12
9. Pure lines varieties have also been used as parents in hybridization for development of superior hybrids in Self pollinated crops.
10. This method is now an integral part of many modern breeding methods.

Differences between Mass and Pureline selections

S. No.	Mass selection	Pureline selection
1	Used both in self and cross pollinated crops	Practiced in self pollinated crops only
2	Large number of plants are selected	Comparatively less number of plants are selected
3	The produce of the selected plants is mixed and sown as such in next year	Produce of individual plants is kept separate and progeny rows are raised next year
4	No control of pollination	Pollination is controlled
5	Variety developed is heterozygous and not uniform	Variety is homozygous homogeneous and uniform
6	Due to heterozygosity the variety deteriorates quickly	Due to homozygosity the variety lasts long
7	The method has to be repeated once in 2-3 years to purify the variety	No need to repeat
8	Wider adaptability due to heterozygosity	Narrow adaptability due to homozygosity
9	No knowledge of science is required. It is more an art.	Knowledge of science and genetics is required
10	Selection within a variety is effective	Selection with in a pureline variety is not effective
11	The variety is relatively difficult to identify	It is relatively easy to identify in seed certification programmes.

Pedigree Method

The "pedigree" may be defined as a description of the ancestors of an individual and it generally goes back to some distant ancestors. It is useful to know the

relationship of two individuals and useful for selection of parents and prediction of outcome of the cross. Given by H. H. Lowe in 1927, in the pedigree method, individual plants are selected from F2 and subsequent generations, and their progenies are tested. During the entire operation a record of all parents to offspring relationships is kept. This is known as **pedigree record**. Individual plant selection is continued till the progenies show no segregation. At this stage the selection is done among the progenies, multi location tests are conducted and released as varieties. In a nutshell, this method is basically to develop a pure line through continuous selection after an hybridization event, with an extra effort of maintaining pedigree record so that to prevent from derailing from the main objectives.

Procedure of pedigree method

1st year: cross is made between all the parents possessing desirable characters.

2nd year: Sow the F_1 seed giving wide spacing so that each F_1 plant produces more seeds. Raise as many F_1 plants as possible to produce large number of F_2 seeds. Harvest in bulk.

3rd year: Grow 2000-10000 plants of F2 giving wide spacing for full expression of the characters in F_2 generation plants. Depending upon the facilities and objectives of the programme about 100-500 superior plants are selected. The selected plants are harvested separately and given serial numbers and description entered in pedigree registers.

4th year: Progeny rowing of F_3 i.e. seeds of one selection plant in one row are space planted along with parents and checks. From superior progeny rows, individual plants with desirable characters are selected (about 50-100 families and about 5 plants in each family and harvested separately). Diseased, lodging and undesirable progenies are discarded.

5th year: F_4 plants raised again as head to row. Desirable plants are selected from desirable rows and harvested separately.

6th year: F_5 plants raised in 3 row plots i.e. seeds of each selected plant sown in 3 rows. By this time many families might have become reasonably homozygous. For comparison, check variety is grown for every 3 or 5 block. Progenies are evaluated for yield and the inferior ones are rejected. The number should be reduced to 25-50. Superior plants from superior progenies are selected. Plants from each progeny are bulked.

7th year: F_6 individual plant progenies are grown in multi-row plots and evaluated. Inferior progenies are rejected and superior progenies are selected. Plants of each progeny are harvested in bulk. Diseased and inferior plants from the progenies are removed.

8th year: F_7 preliminary yield trial with 3 or more replications is conducted to identify superior lines. The progenies are evaluated for many characters including yield. Standard commercial varieties must be included as checks. Two to five outstanding lines are selected and advanced to coordinated yield trials.

9th, 10th and 11th year: selected lines are tested in several localities for 2 or 3 years for adaptation tests. Lines are evaluated for all characters mainly yield and disease resistance. A-line that is superior to commercial variety in yield and other characters are selected.

11th and 12th year: Selected superior lines is named, multiplied and released as a new variety. Number of year can be reduced if generations are advanced during off seasons either in greenhouse or under irrigated conditions.

Merits of pedigree method

1. It gives maximum opportunity for the breeder to use his skill and judgement in selection of plants
2. It is well suited for the improvement of characters which can be easily identified and are simply inherited.
3. Transgressive segregation for yield and other quantitative characters may be recovered.

4. Information about the inheritance of characters and pedigree can be obtained.
5. Inferior plants and progenies are eliminated in early generations.
6. It takes less time than bulk method to develop new variety.
7. Selection is based on phenotypic as well as genotypic selection.
8. A high degree of genetic purity is produced in the cultivar.

Demerits of pedigree method

1. Valuable genotypes may be lost in early generations, if sufficient skill and knowledge are lacking in the breeder, at the time of selection.
2. The selected material becomes so large that handling of the same becomes very difficult, but now modern research plot equipment for planting and harvesting allow complex operation and record taking to be conducted easily.
3. No opportunity for natural selection
4. Difficult to handle many crosses
5. Maintenance of records, selections, growing progeny rows etc are time consuming and laborious.
6. Keeping in view all of above, qualitative traits are best suited as compared to quantitative characters.
7. If only one growing season is possible than it is a very long procedure.

Achievements

Large numbers of varieties have been developed by pedigree method in many crops. A few examples are:

1. **Wheat** – NP-52, 120,125, 700 and 800 series, Janak and Pratap
2. **Rice**- ADT-25, Jaya, Padma
3. **Cotton**-Vikash, Anjali, Lakshmi, Digvijay,
4. **Sorghum**-Co 18, Rs 610,
5. **Tobacco**-NP 222

6. **Tomato**- Pusa early dwarf

Modification of pedigree method

Some specific ways in which pedigree method may be modified are:-

1. The number of plants to select at each step may be modified according to species, the breeding objective and the genetics of the trait of interest, as well as the experience of the breeder with the crop and also the resource available for breeding programme
2. The depth and detail of record kept are at the sole discretion of breeder.
3. Off season planting (e.g. winter nurseries), use of greenhouse and thus having multiple planting per year (wherever possible) are ways of speeding up the breeding process.

Bulk method

The bulk method was first proposed by Nilsson Ehle in 1908. This method is also known as mass method 'or' Population method of breeding. Differs from pedigree in handling of generation. Each segregating generation is grown at normal commercial scale and superior are selected. No record of ancestry is kept. During the early generation, natural selection tends to eliminate plants with poor survival rates. Later on two types of artificial selections also get applied such as manual destruction of undesirable plants and mass techniques such as harvesting for early maturing plants or use of screen to select for greater seed size and so on.

Biggest advantage of bulk method is that it gives breeder freedom to handle very large populations inexpensively.

Procedure of bulk population method

1. **Year 1:** P_1 X P_2 (cross between desired parents) and F_1 harvested (nearly 50-100 plants)

2. **Year 2:** Gen. F_1 grown selfed (naturally), harvested and bulked F_2 (2000-3000).
3. **Year 3**: Gen. F_2 grown, selfed, harvested F_3 (2000-3000 plants)
4. **Year 4:** Gen. F_3 grown at comm. rate, selfed and harvested and bulked F_4
5. **Year 5-7:** Gen. F_4-F_6 grown, harvested and bulked
6. **Year 8:** 10% of Gen. F_7 grown, with progeny rowing and artificial selection done
7. **Year 9:** 10% of Gen. F_8 grown with artificial selection done
8. **Year 10:** Prelim. Yield trial with 10% of gen. F_9 done (usually number is 30-60).
9. **Year 11:** Advanced yield trial with F_{10} - F_{12} at five multiple locations and release of commercial cultivar

Application of Bulk Population

1. Most suitable for breeding species that are normally closely spaced in production e.g. small grains- wheat and barley, and bean (soybean)
2. Breeder may screen bulk population under different natural environment to increase broad adaptation e.g. soil salinity, soil acidity, disease resistance, winter kill but degree of pressure should not be to the tune of killing otherwise superior genotypes

Advantages of bulk breeding method

1. Simple, convenient to conduct, less labour intensive and less expensive in early generation
2. Natural selection increases frequency of desirable genotypes by the end of bulking period.
3. It allows large amount of material to be handled and thus breeder gets a free hand to make and evaluate more crosses.

4. The cultivar we have at the end of the programme is also adapted to environment as an additional benefit as it has gone through years of natural selection.
5. Single plant selection is made when plants are more homozygous (beyond F_6) and thus it improves the value of bulk breeding method.

Disadvantages of bulk breeding method

1. Superior genotypes may be lost to extreme natural selection pressure while undesirable genotypes may develop mechanism to resist Abiotic and biotic stress and thus are advanced during early generation time.
2. It is not suited to species that are widely spaced in normal production plots (because then bulking will have repressive effects on the flowering and seed setting.
3. Genetic characters of population are difficult to ascertain from one generation to the next as no record is maintained.
4. Genotypes are not equally represented in each generation because all the plants in one generation are not advanced to the next level of screening. Improper sampling after F_6 may lead to genetic drift.
5. The procedure is lengthy and we cannot take the advantage of off-season planting because selecting in off-season nurseries and in greenhouses may favour genotypes that are undesirable in the production region and in any case because we are looking for natural selection, off-season planting goes against the idea of this method.

Achievements of bulk method

The method has been used to a limited extent is Barley breeding in U.S.A. and more than 50 varieties were developed. They are: ARIVAL, BEECHER, GLACIER, and GEM.

Single Seed Descent

- Proposed by C.H. Goulden in 1941.
- Method born out of a need to speed up the breeding programme by rapidly inbreeding a population prior to beginning individual plant selection and evaluation, while reducing a loss of genotypes during segregating generations.
- Method is to harvest a single seed per plant aka modified pedigree method
- Widely used in soybean

Key features:

- The method allows the breeder to advance the maximum number of F_2 plants through the F5 generation by either randomly select one seed per plant in early segregating stages or enforcing the plant under artificial conditions to reduce flower size, promote cleistogamy and thus increase chances of fewer seed per plant getting matured. Thus this method is good for legumes, small grains; especially those that are can tolerate close planting and still produce at least one mature seed per plant. Thus species that can be forced to mature rapidly are suitable for breeding by this method.

Procedure

1. **Year 1:** P_1*P_2; F_1 harvested (50-100)
2. **Year 2**: all the F_1 grown in green house at very close spacing
3. **Year 3**: 2000-3000 F_2 grown in less spacing and at maturity single seed/plant is harvested and bulked as F_3
4. **Year 4**: 2000-3000 F_3 grown as year 3 and single seed per plant is harvested as F_4
5. **Year 5**: repeat with F_4
6. **Year 6-8:** prelim yield trial

7. **Year 9-11**: Advance yield trials and most superior line from F_{11}/F_{12} is released as commercial cultivar.

Advantages

1. Easy (done in green houses, off season makes it rapid and easy)
2. Small space is required
3. Duration is reduced.
4. Every plant is from every different F_2 plant, so each plant is one genotype.
5. Natural selection has no effect so, this kind of breeding can be done indirectly in a place away from target site of production.

Disadvantages

1. Natural selection has no effect and hence no benefit from possible positive impact
2. Selection is phenotypic or random
3. If the single seed fails to germinate or if the plants fail to set seed, it thus prohibits every F_2 from being represented in subsequent population.
4. The assumption that single seed represents genetic base of each F_2 genotype may not be true.

Mutation breeding

Factors affecting the success of mutation breeding:

1. **Clear objective:** lesser the number of traits, better it is.
2. **Efficient screening method:** as one has to examine large population to find mutants
3. **Proper choice of mutagen, and type of tissue and methods of treatment:** this refers to soft or dry tissue to be used for mutation breeding and accordingly most suitable mutagen.

4. **Dose rate:** For every different system, experimentally derived dose rate has to be enumerated for effective mutation breeding.
5. **Proper experimental condition:** such as oxygen enhancement ratio where higher the oxygen more is the chances of mutation occurring in cells.

Certain consideration in mutation breeding:

1. There can be primary as well as secondary effect in mutation breeding where primary mutation is basically the mutation which is the desired mutation and secondary is a mutation in some other unconcerned traits.
2. SPCs being homozygous are easy to handle in identifying mutant as compared to CPCs.
3. Seed treatment should be in bulk for having sufficient first generation plant (M_1) which will then be sufficient to produce a larger population of M_2. This is important because one of the most common side effects of mutations is sterility, so a large population of M_2 is always desirable.
4. Seed is multi-cellular and hence a mutation in a single cell will give rise to a chimeric plant, thus an M_1 plant is subject to a competition between mutated and normal tissue during the vegetative phase (diplontic) as well as competition between mutated and normal pollen during reproductive phase (haplontic).
5. As compared to conventional breeding approaches where F_1 plants from inbred lines are genetically identical to either of their parents but M_1 plants may due to mutations, characters which are totally new and not present in either of the parent and therefore M_1 plants are handled as a segregating populations and receives treatment equal to F_2, thus $M_1=F_2$ in mutation breeding.

CV selection strategies when mutagenesis is the breeding method:

1. **Pedigree method**: Each M_1 is harvested separately, progeny rowed and each and every individual upto M_4 is grown with a record of ancestry kept. Beyond M_4, the selected individuals are sent for preliminary and advanced trials and released as a commercial cultivar.
2. **Bulk Method**: Similar to previous bulk method, wherein every new generation is bulked time and again. The more segregation is done, the more mutant we will see and accordingly finer the population will come out. So, prolonging segregation is the key.
3. **Single seed descent**: Similar to previously mentioned approach. Single M_2 seeds are taken for advancing but risk factors are very high.

Applications of Mutation Breeding

Mutation breeding has been used for improving both oligogenic as well as polygenic characters. Mutagenesis has been used to improve morphological and physiological characters including yielding ability. Various applications of mutation breeding are:

1. Induction of desirable mutant alleles which may not be available in the germplasm
2. It is useful in improving specific characteristics of a well adapted high yielding variety.
3. Mutagenesis has been successfully used to improve various quantitative characters including yield.
4. F_1 hybrids from inter-varietal crosses may be treated with mutagens in order to increase genetic variability by inducing mutation and to facilitate recombination of linked genes.
5. Irradiation of inter-specific (distant) hybrids has been done to produce translocations.

Advantages

1. Mutation creates inexhaustible variation.
2. When no improvement is possible this method has to be adopted.

Limitations

1. Frequency of desirable mutations is very low about 0.1 percent. To detect the desirable one in M_2 considerable time, labour & other resources are to be employed.
2. Large number of segregating population are needed, it is like finding needle in a haystack and to screen large population, efficient quick and inexpensive selection techniques are needed.
3. **Associated side effects**: Desirable mutations may be associated with undesirable side effects and deformities due to other mutations thus extending the mutation breeding programme.
4. **Recessivity of mutations**: Detection of recessive mutations in polyploids and clones is difficult and larger doses of mutagen have to be applied and larger populations are to be grown.
5. **Random nature of mutation**: conventional mutagenesis is unpredictable and cannot be directed to specific genes. Thus want of targeted mutation in specific genes has lead to modern biotechnology tools and techniques to perform site directed mutagenesis.

Achievements

1. **Disease resistance** e.g. Verticillium wilt resistance in peppermint, Victorial blight resistance in barley, downy mildew in pearl millet.
2. **Modification of plant structure** e.g. bush habot in dry bean, dwarf mutants in wheat and rice
3. **Nutritional quality augmentation:** opaque/floury endosperm mutant in maize.

4. **Chemical composition alteration:** e.g. low euricic acid mutants of rape seed.
5. **Male sterility:** for use in hybrid breeding in various crops
6. **Horticultural variants:** development of various floral mutants (*Petunia* sp.)
7. Breeding of asexually propagated species
8. Development of genetic stock

Major examples of mutation breeding

a) Natural mutants:

1. Rice: GFB 24 – arose as a mutant from Konamani variety Dee – Gee – Woo –Gen – Arose as a mutant from rice in China MTU 20 – arose as a mutant from MTU-3
2. Sorghum Co. 18 – arose as a mutant from Co. 2
3. Cotton: DB 3-12 from *G. heroaccum* variety Western 1

b) Induced mutants:

1. **Tobacco**: Cultivar Chlorina (first comm. Cv, X-Rays mutagen)
2. **Rice**: Jagannath-gamma ray induced mutant from T. 141
3. **Wheat**: Sarbati Sonora Gamma radiation from Sonora 64, NP 836 mutants through irradiation from NP 709.
4. **Cotton**: Indore 2 Induced from Malwa upland 4, MLU 7 gamma ray induced mutant from culture 1143 **EE**, MLU 10 gamma ray induced mutant from MLU 4.
5. **Mustard**: Primaxwhicte (1950), Summer Pope seed Regina I (1953).
6. **Sugarcane**: Co. 8152 gamma ray induced mutant from Co. 527.
7. **Groundnut**: NC 4.
8. **Castor**: Aruna (NPH1) – Fast neutrons induced mutant from HC 6.

Use of Polyploidy in plant breeding

The somatic chromosome number of any species, whether diploid or polyploid, is designated as 2n, and the chromosome number of gametes is denoted as n. An individual carrying the gametic chromosome number, n, is known as haploid.

A monoploid, on the other hand, has the basic chromosome number, x. In a diploid species, n=x; one x constitutes a genome or chromosome complement. The different chromosomes of a single genome are distinct from each other in morphology and or gene content and homology; members of a single genome do not show a tendency of pairing with each other.

Thus a diploid species has two, a triploid has 3 and a tetraploid has 4 genomes and so on.

In euploids, the chromosome number is an exact multiple of the basic or genomic number. Euploidy is more commonly known as polyploidy.

When all the genomes present in a polyploidy species are identical, it is known as autopolyploid and the situation is termed as auto polyploidy.

In the case of allopolyploids, two or more distinct genomes arepresent. Euploids may have 3 (triploid), 4 (tetraploid), 5 (pentaploid), or more genomes making up their somatic chromosome number.

In case of autopolyploidy, they are known asautotriploid, autotertaploid, autopentaploid, and so on, while in the case of allopolyploidy they are termed as allotriploid, allotetraploid, allopentaploid, etc. Amphidiploid is an allopolyploid that has two copies of each genome present in itand, as a consequence, behaves as a diploid during meiosis. A segmental allopolyploid contains two or more genomes, which are identical with each other, except for some minor differences.

Breeding Autopolyploids

Origin and production of doubled chromosome numbers:

1. **Spontaneous**: chromosome doubling occurs occasionally in somatic tissues and unreduced gametes are produced in low frequencies.
2. **Production of adventitious buds**: decapitation in some plants leads to callus development at the cut ends of the stem. Such a callus has some polyploid cells and some of the shoot buds regenerated from the callus may be polyploid. Insolanaceae, 6-36% of adventitious buds are tetraplods. The frequency of ploypoid buds may be increased by the application of 1% IAA at the cut ends as it promotes callus development.
3. **Treatment with physical agents:** Heat or cold treatment centrifugation, x-ray orgamma ray irradiation may produce polyploids. Exposing the plants or ears of maize to a temperature of 38-45°C at the time of the first division of zygote produce 2-5 % tetraploid progeny.
4. **Regeneration in vitro**: polyploidy is a common feature of the cells cultured in-vitro.
5. **Colchicine treatment**: Colchicine treatment is the most effective and the most widely used treatment for chromosome doubling. Chromosome doubling reduces the risk of meiotic complications, and thus increases chances of fertile autopolyploids.

Morphological and cytological features of autopolyploids

1. Polyploids have larger cell size than diploids. Guard cells of stomata are larger the number of stomata per unit area is less in polyploids than diploids.
2. Pollen grains of polyploids are generally larger than those of the corresponding diploids.
3. Polyploids are generally slower in growth and later in flowering.
4. Polyploids usually have larger and thicker leaves, and larger flowers and fruits which are usually less in number than in diploids.

5. Polyploids generally show reduced fertility due to irregularities during meiosis and due to genotypic imbalance leading to physiological disturbances.
6. In many cases, autopolyploidy leads to increased vigour and vegetative growth.
7. Different species have different levels of optimum ploidy. For sugar beet the optimum level is 3x, sweet potato 6x while for timothy grass it is between 8-10x.
8. Autopolyploids generally have a lower dry matter content than diploids.

Application of Autopolyploidy in Crop improvement

Triploids

Triploids are produced by hybridization between tetraploid and diploid strains. They are generally highly sterile, except in a few cases. This feature is useful in the production of seedless watermelons. In certain species, they may be more vigorous than the normal diploids, e.g., in sugar beets.

Seedless watermelons are produced by crossing tetraploid (4x, used as female) and diploid (2x, used as male) lines, since the reciprocal cross (2x x 4x) is not successful. The triploid plants do not produce true seeds; almost all the seeds are small, white rudimentary structures like cucumber (*Cucumissativus*) seeds. There are several problem viz. genetic instability of 4x lines, irregular fruit shape, a tendency towards hollowness of fruits, production of empty seeds and the labour involved in triploid seed production.

1. **Triploid Sugar beets**: Among root crops triploid sugar beets apparently represent the optimum level of polyploidy because 3n plants have longer roots than diploid and also yield more sugar per unit area.

2. **Tetraploid Rye**: The advantage of tetraploid over its diploid counterpart are large kernel size, superior ability to emerge under adverse condition and higher protein content. Tetraploid rye varieties have been released for cultivation e.g. Double steel, Tetra petkus.

Limitations of autoployploidy

1. Larger size autopolyploids generally contain more water (gigas effect) and produce less dry matter content than diploids.

2. High sterility with poor seed setting is observed

3. Due to complex segregation, progress through selection is slow.

4. Monoploids and triploids cannot be maintained except through clonal propagation

5. The varieties cannot be produced at will

6. Effects of autopolyploidy cannot be predicted.

Allopolyploidy

Allopolyploids have genomes from two or more species production of allopolyploids has attracted considerable attention; the aim almost always was creation of new species. Some success has been evident from the emergence of triticale, *Raphanobrassica* and allopolyploids of forage grasses.

Morphological and cytological features of allopolyploids

1. Allopolyploids combine the morphological and physiological characteristics of the parent species but it is very difficult to predict the precise combination of characters that would appear in the new species.
2. Many allopolyploids are apomictic as in Tulips, Solanum.
3. The chromosome pairing in the new species depends upon the similarities between the chromosomes of the parental species. Chromosomes with such similarities are known as homeologous chromosomes. After chromosome doubling, the allopolyploid would have two homeologous chromosomes for each chromosome present in the F_1 hybrid, comparable to the diploid species. Such allopolyploid is referred as amphidiploid or Allotetraploid.
4. Fertility of allopolyploids can be improved by hybridization and selection.

Application of allopolyploidy in crop improvement

1. Utilization as a Bridge species: Amphidiploids serve as a bridge in transfer of characters from one species to a related species, generally from a wild species to cultivated species. Creation of new crop species as in Triticale, *Raphanobrassica.*
2. Widening the genetic base of existing Allopolyploids: The genetic base of some natural allopolyploids may be narrow, and it may be useful to introduce variability in such cases by producing the allopolyploids afresh from their parental species.

Limitations of Allopolyploidy

1. The effects of allopolyploidy cannot be predicted. The allopolyploids have some features from both the parental species, but these features may be the undesirable ones, e.g., *Raphanobrassica,* or the desirable ones, e.g., *Triticale.*
2. Newly synthesized allopolyploids have many defects, e.g., low fertility, cytogenetic and genetic instability, other undesirable features etc.
3. The synthetic allopolyploids have to be improved through extensive breeding at the polyploidy level. This involves considerable time, labour and other resources.
4. Only a small proportion of allopolyploids are promising; a vast majority of them are valueless for agricultural purposes.

Aneuploidy

Aneuploidy is referred to individuals with an incomplete set of chromosome that is equivalent to the euploid number plus or minus one or more specific chromosome. As in, normal set if is 2n then 2n-1, 2n-2, 2n+1 are referred to as monosomy, nullisomy, and trisomy respectively.

Heterosis breeding and Male Sterility

What is a hybrid cultivar?

The F_1 offspring of a planned cross between inbred lines, CVs, clones or population. Depending on the breeding approach, arents can be two to many with critical requirement of un-identical parents and it is this divergence that gives hybrids their superior performance and this superior performance is due to the exploitation of the phenomenon of heterosis or hybrid vigour.

Concept of hybrid vigour and inbreeding depression

Hybrid vigour may be defined as the increase in size, hardiness, growth, temperature tolerance, vigour, fertility, and overall productivity or performance of a hybrid plant over the mid parent value (average performance of two parents). Although, practically hybrid vigour is defined as that greatly exceeds the better or higher parents in a cross.

Calculated as Hybrid vigour (%) = [[F_1-(P1+P2/2]X100] divided by (P1+P2)/2.

G.H. Shull coined a synonymously popular term, **heterosis.**

Heterosis, occurs when two inbred lines of out bred species are crossed or simply pure lines of two very diverse parents are crossed. Heterosis, though widespread in plants, is not uniformly manifested in all species and for all the traits. It is manifested at a higher intensity in traits that have fitnessvalue, and also more frequently in cross pollinated species than self pollinated species. Every cross uses heterosis as an advantage to some extent.

Inbreeding depression: Reduction in fitness (depression) due to forced selfing (inbreeding) is Inbreeding depression. It is opposite of heterosis. Reduction in fitness is usually manifested as a reduction in vigour, fertility and productivity. Effects of inbreeding are more severe in early generation (5-8). Just like

heterosis, inbreeding depression is not uniformly manifested in plants. Some tolerant inbreeding depression while some are intolerant.

Genetic basis of heterosis has been explained by two theories, one is Dominant hypothesis and other is over dominant hypothesis. Both are partially valid and partially fail to explain certain heterotic observations. While the dominant hypothesis says that it is the dominant loci rather than heterozygosity which causesheterosis. The recessive genes are deleterious while dominant are superior. Inbreeding or selfing results into loss of vigour because it ends up in homozygous recessive condition for certain loci, crossing restores the hybrid vigour as the resultant progeny become heterozygous for most of the lociand the effect of deleterious recessive gene are masked by their dominant alleles. Another theory that is over dominant hypothesis says that both dominant and recessive alleles have their respective contribution, and the hybrid vigour is due to the metabolic advantage of gene products by these two different kind of alleles, thus heterozygotes are better buffered against disturbances and thus are more vigourous.

Fixation of Heterosis

For commercial application, fixation is necessary. Use of techniques or mechanisms to ensure that the heterotic gene combinations produced in a heterotic individual remain intact in the subsequent generation is known as fixation of heterosis. There are three ways of fixing heterosis:

1. Vegetative reproduction
2. Apomixes
3. Balanced lethal system, where two recessive lethal genes are linked in repulsion phase. In such situations only heterozygotes can survive. So it provides fixation of heterosis, although considerable no. of ovule sterility cases will rise.

Achievements of heterosis

Heterosis is utilized in two ways, viz. either fully or partially. Full heterosis is exploited through the development of hybrids and partial hybrids are exploited by developing synthetics and composites. In India, heterosis has been commercially exploited in maize, Jowar, Bajra, cotton, arhar, some vegetables and fruits tress like coconut. It is more exploited in cross pollinated species some examples are Ganga 2 and Ganga 5 in Maize, Varalaxmi and Dhanalaxmi in Cotton, GCH2 and GCH3 in castor, BSH1 in sunflower, HB9, BJ104 in pearl millet. In self pollinated species, heterosis has limited application because hybrid seeds production at large scale is very difficult. Even though in some crops it has been exploited for example hybrid tomato in tomato, Vijay and pusakranti in brinjal and hybrid C in jute.

Merits and demerits

Merits of heterosis

1. Higher yield potential and more uniform.
2. It is possible to reconstitute the hybrid with some genotype which is not possible in case of composite varieties.

Demerits of heterosis

1. Fresh seeds have to be produced every year because in F2 the hybrid produces various types due to segregation and recombination. So, farmers have to purchase fresh seed every year.
2. The seed of hybrid variety is costlier
3. Cultivation of hybrids requires more input like irrigation, fertilizers etc to exploit their full potential.

Even though it has some demerits it is one of the most significant and accepted breeding process.

Use of Backcross in plant breeding

Backcross refers to crossing of F_1 with either of its parents. A system of breeding in which repeated backcrosses are made to transfer a specific character to a well adapted variety for which the variety is deficient is referred to as backcross breeding. The main objective of backcross breeding is to improve one or two specific defects of a high yielding variety (known as elite).

Breeding Procedure

In backcross breeding procedure the most important step is in selection of parents. There are two parents, one is called recurrent (i.e. the elite variety) and other is non-recurrent. The recurrent parent must be the most popular variety of the area. The non-recurrent parent is selected for the character that is to be improved in the recurrent parent. *The plan of backcross method would depend upon whether the gene being transferred is recessive or dominant.*

Case I: Transfer of dominant gene

Suppose wilt resistance in cotton is controlled by a dominant gene R which is present in strain B. So, this parent will be called non-recurrent parent. The adapted variety A will be used as recurrent parent. Generally 6-8 backcrosses are sufficient to obtain almost all (99.9%) gene of adapted variety along with resistance gene. The following figure shows the procedure of transfer of dominant gene through backcrosses.

Strain B (wild type or non-elite, non-recurrent parent, RR) x Strain A (elite, recurrent parent, rr)

↓

Rr (F1) x Strain A (rr).....BC1

↓

rr;Rr x rr (recurrent parent).....BC2

This will go on likewise for 6-8 generations

↓

After this, identical plant progenies are grown and selected, individual plants are grown and homozygous progenies are harvested.

↓

Primary yield trials, then replicate of yield trials, seed multiplication and distribution.

Case II: transfer of recessive gene

Suppose wilt resistance in cotton is governed by a recessive gene (r), in such a case, the progeny of each backcross will segregate into two genotypes (RR and Rr) which cannot be identified. Therefore, it is necessary to self the population after each backcross to obtain resistant homozygous recessive plants (rr). It means here each backcross is followed by one selfing and screening for rr types. Rest procedure is similar to procedure for transfer of dominant gene as mentioned in **Case I.**

Applications of Backcross as a tool in plant breeding

1. For development of disease resistance varieties (example: Digvijay, V797, Kalyan varieties of Cotton)
2. Intervarital transfer of quantitative characters may also be done through backcross, if they have high heritability. Apart from this, the quantitative characters must be in more intense form in the non-recurrent parent then it is desirable in the new variety.
3. Transfer of cytoplasm
4. Backcross method has also been used for inter-specific gene transfer and development of multiline varieties in SPC.

Merits of BCBM

1. This method retains all desirable characters of a popular adapted variety and replaces undesirable alleles at a particular locus.
2. Point 1 above implies that defects can be removed without affecting its performance and adaptability, which is often preferred by farmers and industries.
3. In this method, this is not necessary to do the extensive yield test because recurrent parent is already known.
4. Much smaller population are needed so, handling becomes easy.
5. This method is extensively used in the development of varieties with multiple disease resistance.

Demerits of BCBM

1. New variety generally not superior than recurrent parent except for one or few characters.
2. It involves a lot of crossing work.
3. Linkage drag, means undesirable characters tightly linked with desirable ones, inhibit the process of backcross.
4. Backcross is very important breeding method for varietal development but there may be case that by the time backcross programme completes, the recurrent parent may be replaced by other sup. Variety. So it may be a limitation in BCBM. So there should be proper planning before the backcross breeding for its success.

Male sterility

Use of Male sterility in plant breeding

First reported by Kolreuter, it is characterised by non-functional pollen grains or anthers while female reproductive part is completely normal. The condition may manifest as absence, extreme scarcity of pollen, malformation or absence of flowers or stamens, failure of pollen to dehisce. Male sterility has been an important out breeding device for the breeding which help prevent self pollination.

Based on the origin of abnormality there are three basic kinds of male sterility:

1. **True male sterility:** Due to lack of male sex organs in male flowers or in bisexual flowers. Sometimes male sex organs are present with abnormal or non-functional microspores leading to pollen abortion. It is mainly of three types: Genetic male sterility (GMS), Cytoplasmic male sterility (CMS), cytoplasmic genetic male sterility (CGMS).
2. **Functional male sterility:** The anthers fail to release their content even though the pollen is fertile.
3. **Induced male sterility:** Breeder induced male sterility by applying gametocides, hormones or by transgenic means. It is a rapid method of developing male sterile lines. This method has some limitation like pollen abortion is incomplete and erratic too because treatment is effective only at a specific stage with lots of side effects. Some examples are Naphthalene Acetic Acid (NAA) in cucurbits, Maleic Hydrazide (MH) in wheat, tomato, cucurbits, Gibberellins in Rice, maize, sunflower and ethrel in Wheat, Rice and sugar beet.

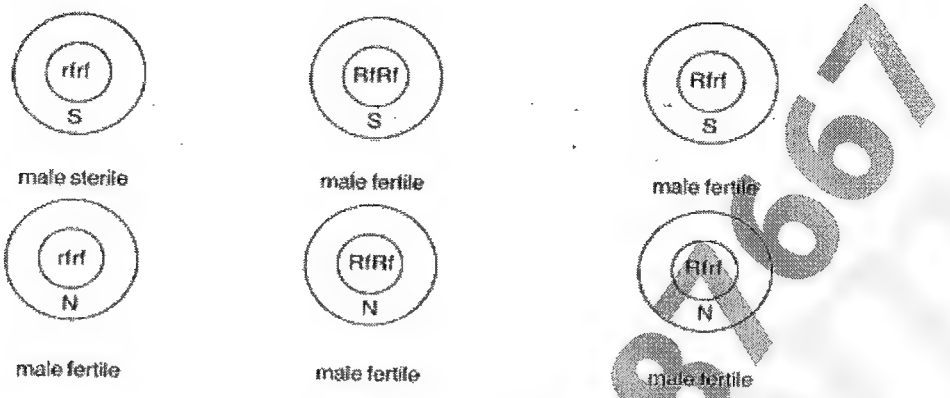
Transgenic male sterility is induced in systems by introgressing genes from other sources usually from microorganisms e.g. transgenic male sterility has

been induced in tobacco and rapeseed by transferring a gene (barnase gene) from Bacillus amyloliquifanciens. Another gene (barstar) suppresses the male sterility gene (barnase) and leads to restoration of fertility.

Genetic male sterility (GMS): The pollen sterility which is caused by nuclear genes is termed as GMS. This sterility has been reported in many crop plants like barley, wheat, maize, cotton, sorghum, cucurbits, tomato and sugar beet. The GMS genes are usually recessive and rarely dominant and in majority of cases, sterility is caused by single gene. It is not widely used for commercial hybrid seed production. GMS may or may not be influenced by temperature and photoperiod. Some examples where GMS has been exploited are watermelon, cotton, wheat and rice.

Cytoplasmic Male Sterility (CMS): Controlled by mitochondrial genes, transmitted only through egg (maternal inheritance), this kind of male sterility is controlled by cytoplasmic genes. CMS is not influenced by environmental factors such as temperature and photoperiod. This systems contains male sterile and male fertile lines. It cannot be utilized for hybrid seed production without the use of restorer line because F_1 seeds produce only male sterile plants. Even though, CMS can be used for development of hybrids in vegetatively propagated crops and ornamental crops where grain is not the economic product. Example where CMS is prevalent are Forage, ornamental plants, Sorghum, Corn, Sugar beet, carrot and flax

Cytoplasmic-Genetic Male Sterility (CGMS): When pollen sterility is controlled by the interaction of cytoplasm and nuclear genes, it is known as CGMS. Discovered by Jones and Devis in 1944 in Onion. This system includes three lines male sterile line, male fertile line or maintainer line and restorer line (so called because of presence of fertility restoring gene in nucleus). CGMS is used commercially to produce hybrid seeds in Maize, Bajra, cotton, rice sunflower, sorghum, onion and Jowar.

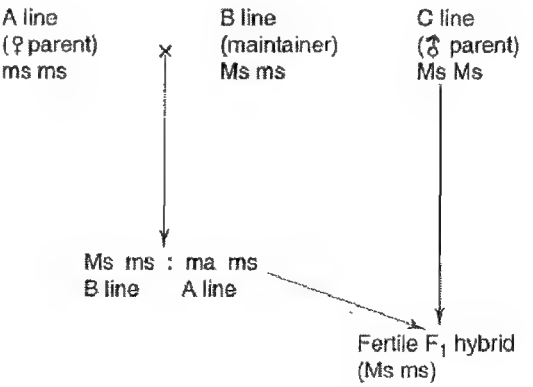


Limitations: it is very difficult to identify the male sterile lines when controlled by nuclear genes before anthesis. So, male sterility cannot be used in those crops in which efficient natural pollen dispersal mechanism is lacking and flower morphology is not suitable for cross pollination.

Detailed discussion of individual types

Nuclear Male Sterility (NMS)

Nuclear male sterility also called as 'genetic or genic' male sterility (GMS), is usually caused by a recessive allele 'ms'. Nuclear male sterility originating through spontaneous mutation is a common phenomenon in nature. It is of wide occurrence in flowering plants and as many as 60 genes for male sterility in maize, 55 in tomato, 10 in cotton and 60 in rice are known (Homer and Palmer, 1995). The dominant allele 'Ms' results in the development of normal anthers and pollen. Therefore, 'ms ms' genotype is male sterile, while 'Ms ms' or 'Ms Ms' are male fertile. It has not been largely used for hybrid seed



production because it is not possible to produce a population of 100 per cent male sterile plants by this method as the maximum percentage of male sterile plants that can be produced is 50%. The completely male sterile plants (ms ms) are crossed with heterozygous (Ms ms) to produce a progeny of Msms and msms out of which 50 % are fertile and that can be crossed with steriles to maintain male sterility. The nuclear male sterility may originate through spontaneous mutation or can be induced through physical or chemical mutagenesis.

Nuclear male sterility and hybrid seed production

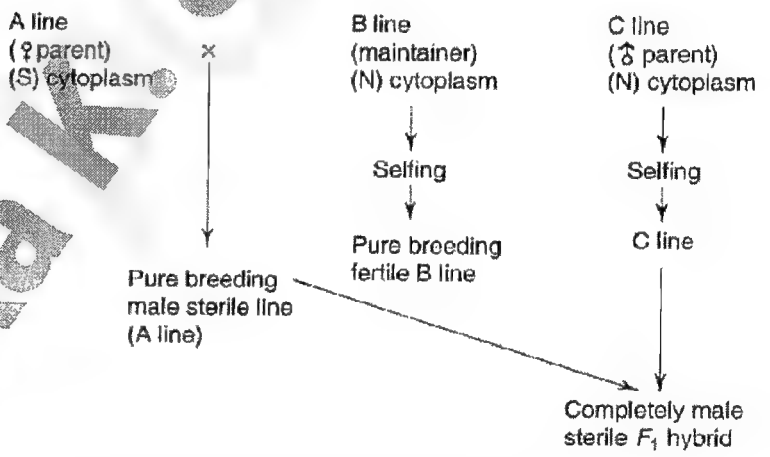
Nuclear male sterility is usually recessive and monogenic as a result of which fertility restoration in the hybrid and crossing scheme are relatively easy. In this case, roguing of fertile heterozygous (Ms ms) plants is essential in the seed production plots. As shown in figure here, pure breeding male sterile lines cannot be maintained unless fertility is restored by a modified environment as in case of photosensitive genetic male sterility system (PGMS) where same line can be made male sterile or fertile under different photoperiodic conditions. Maintenance of male sterile (A) line needs identification of heterozygous fertile plants (B) which are used to pollinate male sterile plants to produce seed that shall segregate for male sterility and fertility in the ratio of 50:50. For hybrid seed production the fertile plants from male sterile line are to be identified and rogued before anthesis. This could be achieved by morphological marker genes closely linked to male sterility locus. Several characters linked with 'ms' gene have been reported as white endosperm in maize, shrunken endosperm in barley, short leaf in sorghum and woolly character in tomato. However, insufficient linkage would result in recombination between marker and male sterility gene as a result of which the marker aided identification becomes invalid. The male sterility conditioned by dominant genes has been reported in cotton, wheat, carrot and oil-seed rape but it cannot be used in hybrid seed production as the F1 hybrid is rendered male sterile. The commercial hybrid seed is produced by crossing the male sterile plants with another line C which is' homozygous for fertility. If seed is the economic part then we have to be sure that recessive male

sterility gene (ms) is not present in the C line. Thus the production of hybrid seeds utilizing nuclear male sterility is more complicated, cost and labour intensive.

Cytoplasmic Male Sterility

The male sterility governed solely by cytoplasmic factors without any dependence on nuclear genes is termed as cytoplasmic male sterility. The Cytoplasmic genes responsible for male sterility are located in the mitochondria and not in the chloroplasts.

Since the offsprings inherit the cytoplasm of only the female parent, so the cross of cytoplasmic male sterile plants leads to completely male sterile progeny. Though cytoplasmic male sterility is a widely used term but such a class may only be reflecting the lack of suitable genes which actually interact with cytoplasm to regulate sterility and fertility. Kaul (1998) expressed that male sterility controlled exclusively by cytoplasmic genes i.e. cytoplasmic male sterility does not exist and is a total misnomer. The true cytoplasmic male sterility should remain uninfluenced by nuclear genes but such stable male sterile types are unknown as fertility restorer genes and maintainer nuclear genes have been detected in many plants (Kaul, 1988). All cytoplasmic male sterility may only be a form of genic-cytoplasmic male sterility which is so labelled till suitable interacting nuclear genes are identified. It may not be improbable that all presently known cytoplasmicgenic male sterility was at one time only cytoplasmic male sterility.

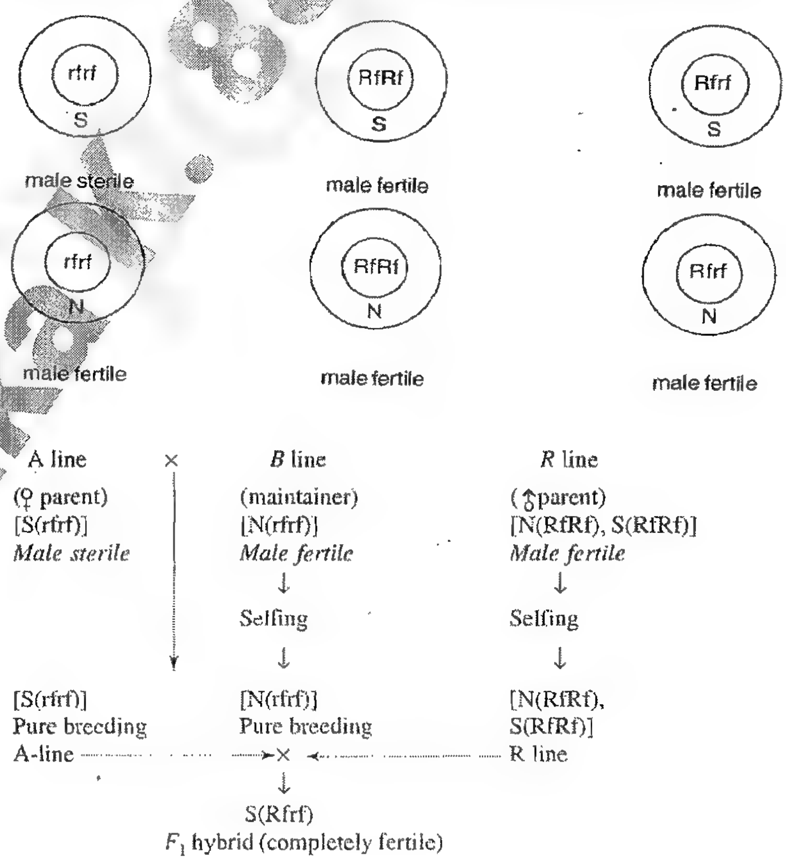


Cytoplasmic Male Sterility and Hybrid Seed Production

In case of cytoplasmic male sterility, the nuclear genes do not play any role and it is based solely on cytoplasmic (mitochondrial) genes transmitted maternally. The hybrids being governed by cytoplasm of female parent are thus always male sterile. Therefore, this system is useful only in crops where seed production is not important. Maintenance of the parental lines and production of hybrid seeds through the use of cytoplasmic male sterility is shown in Figure above.

Cytoplasmic-Genetic Male Sterility

Cytoplasmic-genetic male sterility results from the interaction among the cytoplasmic (mitochondrial) and nuclear genes. Cytoplasmic genetic male sterility (CGMS) and cytoplasmic male sterility (CMS) are used interchangeably. It has been reported in several hundred species (Kaul, 1988) and is being widely used for production of hybrid seed both in self- and cross-pollinated crops (sorghum, sunflower, pearl millet, maize, rice and oilseed rape). In this system, a specific mutation in the mitochondrial DNA (mtDNA) in combination with proper nuclear background leads to the failure of mature pollen formation. In some cases, the nuclear genes often called fertility restoration genes can



compensate for the cytoplasmic mutation and normal pollen formation occurs. Therefore the expression of mitochondrial (cytoplasmic) male sterility genes is governed by the type (dominant/recessive) of nuclear fertility restoring genes. The fertility restoring alleles have been represented by symbols like Rf (fertility restoring) in wheat, sunflower and corn, and Ms (male fertile) in onions, sorghum and pearl millet. Different combinations of sterile (S), normal (N) cytoplasms and fertility restoring genes determining the sterility/fertility of the plant are given in figure below.

Use of CGMS for commercial hybrid seed production

Normal (N) cytoplasm, irrespective of the gene in the nucleus results in a fertile plant. Sterile (S) cytoplasm operates only when both the alleles of fertility restoring nuclear gene are recessive (rfrf). Whereas, in the presence of dominant fertility restoring allele, the sterile cytoplasm becomes inoperative and the plant is normal and fertile. Generally the use of CGMS for commercial hybrid seed production involves the use of three breeding lines, namely:

1. **A-line:** CGMS-line or male sterile containing sterile cytoplasm and homozygous nuclear male sterility gene [S(rfrf)],
2. **B-line:** maintainer, cytoplasmic male fertile line carrying normal cytoplasm but nuclear gene for sterility [N(rfrf)] and is isogenic to A-line. It is used to multiply the CGMS line.
3. **R-line:** Restorer line, male parent, carries gene(s) [N(RfRf), S(RfRf)] which masks the expression of CGMS trait and restores fertility to the F1 hybrid.

Cytoplasmic-genetic male sterility has already found large scale application in the production of hybrid seeds especially in crops like sorghum, sugar beet, corn, onion and carrot.

Use of Apomixis in plant breeding

Apomixis is a clonal reproduction through seed and the off-springs are genetically identical to the mother plant. In apomixes embryo development takes place without fertilization. Usually apomixes found in plants are facultative but in some species like citrus, apple apomixes is obligate.

Type of Apomixis

Gametophytic and Sporophytic are the two main types. In gametophytic there are further two types apogamy and parthenogenesis. Apogamy is the origin of embryo from either synergids or antipodal cells of embryo sac and depending on from where it is coming from it can be haploid or diploid apogamy. Parthenogenesis is the development of embryo from egg without fertilisation.

Sporophytic apomixes is of two types, apospory and adventives embryony. Apospory is when the first diploid cell of ovule lying outside the embryo sac development into another embryo sac without reduction. This diploid embryo thus develops without fertilisation. Adventive embryony is when the diploid cells of ovules outside the embryo sac belonging to either nucellus or integuments are involved in development in embryo.

Uses in plant breeding

Apomixis has several useful application in plant breeding. Some of them are following:

1. **Rapid production of pure lines**: Haploid parthenogenesis gives rise to haploid plants which upon colchicines treatment will produce diploid pure lines that can be used in plant breeding programme.

2. **Maintenance of superior variety**: Apomixis is useful in maintaining the characteristics of mother plant from generation to generation.
3. **Conservation of heterosis:** hybrid vigour may be conserved for many generations by using recurrent apomixes.
4. **Elimination of virus infection**: in apomixes type of vegetative propagation, the problem of virus infection is eliminated because of double barrier of meristem and mega-gametophyte.
5. **Progeny test:** if obligate apomixes is found then there is no need of progeny test for genetic stability. So, apomixes simplify some breeding processes.
6. **Fixing genetic markers**: apomixes is also used in identification of commercial cultivar because it fixes genetic markers.
7. **Commercial seed production:** Apomixis simplifies the commercial see production because it eliminates the contamination due to our breeding.
8. In citrus, apomixes is useful in uniform root stock production through adventives embryony which has indirect application in plant breeding.

Apomixis which was earlier considered as evolutionary dead end and an impediment to plant breeding, now it has wide application in breeding. Plant breeders are making effort to introduce this trait into many commercial and field crops through various methods like recombinant DNA technology. In future with proper research and development the application of apomixes in plant breeding will become more significant.

Wide hybridization or distant hybridization

Introduction

When individuals from two distinct species of the same genera or of two genera are crossed, it is known as **inter specific or inter-generic hybridization** respectively, e.g. *Oryzasativa* x *O. Perennis or* Wheat x rye. Hybridization between individuals from different species belonging to the same genusor two different genera is termed as distant hybridization or wide hybridization, and such crosses are known as distant crosses or wide crosses. The first distant hybridization was a hybrid between carnation (*Dianthuscaryophyllus*) and sweet willian (*Dianthus barbatus*) by Thomas Fairchild in 1717 and the hybrid is called as ***Fairchild's mule.*** An interesting inter-generic hybrid *Raphano brassica* was an amphidiploid cross between radish and cabbage but it was useless. Another first inter-generic hybrid with a great potential was triticale (*Triticum* x *Secale.*

Objectives of such wide crosses

There are many objectives out of which most important are as follows:

1. To transfer some desirable character from wild relatives that is not available in cultivated varieties. For example,
    a. Many disease resistance and, insect resistance genes
    b. Wider adaptability: (i.e. drought-resistance, cold tolerance etc.
    c. Quality improvement (as in Cotton (fibre) Tobacco (leaf)
    d. Yield improvement (as in Oats, Tobacco, Maize, S. cane)
    e. Other characters (as in CMS, Earliness, dwarfness)
2. Exploitation of heterosis in vegetatively propagated /ornamental crops.

3. Creation of Novel genotypes: New species or F_1 hybrids which are otherwise nonexistent in nature.

Barriers To The Production Of Distant Hybrids: the main barriers in hybrids from distant crosses are due to:

a. Failure of zygote formation/Cross incompatibility
b. Failure of zygote development/Hybrid in viability
c. Failure of F1 seedling development/Hybrid sterility

A variety of mechanisms may be responsible for each of these three difficulties:

1. Failure of zygote formation/cross incompatibility.

Inability of the functional pollens of one species or genera to effect fertilization of the female gametes of another species or genera is referred to as **cross incompatibility**. It may be due to

1. Failure of fertilization, because the pollen may not germinate.

2. Pollen tube is unable to reach to embryo sac and hence sperms are not available for fertilization

3. Pollen tube may burst in the style of another species as in the case of *Datura.*

4. The style of the female parent may be longer than the usual length of the pollen tube growth therefore the pollen does not reach the embryo sac as in *Zea mays* and *Tripsacum sp.*

5. Pollen tubes of polyploidy species are usually thicker than those of diploid species.

6. When a diploid is used as female and a polyploidy as male, the polyploidy pollen tube grows at a slower rate in the diploid style than it would be in a polyploid style.

These barriers are known as pre-fertilization barriers.

Techniques to make wide crosses successful:

1. Removal or scarification of stigma

2. Using short styled parent as female.

3. Using the diploid species as the male parent.

2. Failure of zygote Development/Hybrid in viability

The inability of a hybrid zygote to grow into a normal embryo under the usual conditions of development is referred to as **hybrid in viability.** This may be due to

1. **Lethal genes:** some species carry a lethal gene, which causes death of the inter-specific hybrid zygote during early embryonic development as in the case of *Aegilopsumbellulata* carries a lethal gene with 3 alleles against diploid wheat.
2. **Genetic Dishormony between the two parental genomes:** The genetic imbalance between the two parental species may cause the death of embryos as in the case of Cotton -G. gossypioides x other G. Sps and Brassica – B. napus x B. oleracea.
3. **Chromosome elimination:** In some cases of distant hybridization, chromosomes are gradually eliminated from the zygote. This generally does not prevent embryo development, but the resulting embryo and the F1 plants obtained from such crosses are not true inter-specific hybrids since they do not have the two parental genomes in full. Generally. Chromosomes from one are successively eliminated due to mitotic irregularities as in the case of *Hordeumbulbosum*x *H. Vulgare, Hordeumbulbosum*x *Triticumaestivum, and Triticumaestivum x Zea mays.*
4. **Incompatible cytoplasm:** Embryo development may be blocked by an incompatibility between cytoplasm of the species used as female and the genome of the species used as male. Such an interaction, more generally, leads to hybrid weakness and male sterility in the hybrids or may sometimes leads to failure of embryo developments.

5. **Endosperm Abortion: Seeds** from a large number of distant crosses are not fully developed and are shrunken due to poorly developed endosperm. Such seeds show poor germination, and may often fail to germinate. When the endosperm development is poor or is blocked, the condition is generally known as endosperm abortion as in the case of Triticum x secale – Triticale, in which case, the endosperm aborts at a much later stage so that a small frequency of viable seed is obtained. Another example is of *Hordiumbulbosum x H. vulgare*– the endosperm aborts at an early stage so that viable seeds are not produced. In such cases of endosperm abortion - **embryo rescue culture** is practiced.

3. Failure of Hybrid seedling development/Hybrid sterility:

Some distant hybrids die during seedling development or even after initiation of flowering. The mechanisms involved in the failure of seedling development most likely involve complementary lethal genes. For instance in cotton-certain inter-specific hybrids appear normal, but die in various stages of seedling growth; some plants die at flowering. Also, inter-specific and inter-generic F_1 hybrids of wheat show both chlorosis and necrosis.

Hybrid sterility: Hybrid sterility refers to the inability of a hybrid to produce viable off spring. The main cause of hybrid sterility is lack of structural homology between the chromosomes of two species.

Techniques for production of distant hybrids

1. **Choice of parents: Genetic** differences exist among parents in a species for cross compatibility. More compatible parents should be selected for use in wide crosses.
2. Pollinating sufficiently large no. of flowers.
3. **Reciprocal crosses: it** is better to attempt reciprocal crosses when distant crosses are not successful. For instance, *Phaseolusaureus* and *p.mungo*are crossable only when *P. aureus* in used as female and *P. mungo*as male.

4. **Bridge crosses:** Some times, two species say 'A' and 'C' do not cross directly. In such case a third species say 'B' which can cross with both 'A' and 'C' is chosen as abridge species. First 'B' is crosses with 'C' and then the amphidiploid is crossed with 'A'. Bridge crosses have been used in Tobacco and wheat. For instance, *Nicotianarepanda* can cross with *N. sylvstis* but not with *N. tabacum N. sylvestris* crosses with both *N. repanda* and *N. tabacum*. For transfer of genes from *N. repanda to N. tabacum; N. sylvestis*is used as bridge species.
5. **Use of pollen mixtures:** Cross incompatibility results due to unfavourable interaction between the protein of pistil and pollen which inhibits normal germination and growth of pollen tube. This problem can be overcome by using the mixture of pollen from compatible (self) and incompatible parents.
6. **Manipulation of pistil:** In some cases, pollen tube is short and style is very long, due to species difference. Thus pollen tube cannot reach ovule to effect fertilization. In such situation either reciprocal cross should be made or the style should be cut to normal size before pollination. This technique is successful in maize –Tripsacum crosses, where maize style remains receptive even after cutting.
7. **Use of growth regulators:** Some times, the pollen tube growth is so slow that the egg cell dies or the flower aborts before the male gametes reach the ovary. In such cases, growth regulators should be used to accelerate the pollen tube growth or to prolong the viability of the pistil. Use of growth regulators such as IAA; NAA; 2,4-D and GA3 etc; are promising in some wide crosses.
8. **Large number of crosses:** The success of seed set is generally very low in wide crosses. Hence, large no. of crosses should be made to obtain crossed seeds.

9. **Protoplast fusion:** The wide crosses can be obtained through protoplast fusion, when it is not possible to produce such crosses through sexual fusion.
10. **Embryo culture:** This technique is being used widely to obtain viable inter-specific or inter-generic hybrids. This is used when hybrid zygote is unable to develop. This technique has been successfully used is *Triticum, Hordeum, Phaseolun, Nicotiana, Gossypium, Lycopersicon, Trifolium, Cucurbita* etc.
11. **Grafting :** Grafting of inter-specific hybrid on to the cultivated species helps in making the cross successful.

Applications of wide hybridization in crop improvement

1. **Alien addition lines**: Carries one chromosome pair from a different species in addition to somatic chromosome complement. For E.g. Disease resistance in Wheat, oats, tobacco.
2. **Alien substitution lines**: has one chromosome pair from different species in place of the chromosome pair of the recipient parent.
3. **Introgression of genes**: Transfer of small chromosome segments with desirable genes. For instance:
   A. Disease resistance: As in the case of Cotton; transfer of black arm disease resistance from *G. arboreium to G. barbadense*
   B. Wider adaptation: As in, cold tolerance has been transferred from wild relatives to Wheat, onion, potato, tomato and grape.
   C. Quality: Oil quality in oil palm was improved by genes from wild relatives.
   D. Changing the mode of reproduction through manipulating self-incompatibility as in the case where self in compatible genes from *B. campestris* were transferred to self compatible *B. napus* for hybrid seed production.
   E. Yield and other morphological characters.
4. Development of New crop species: e.g. Triticale

5. Utilization as New hybrid varieties: e.g. F_1 hybrids in cotton i.e. Varalaxmi cotton (*G.hirsutum* x *G. barbadense*); another e.g. is of Sugarcane where all the present day commercial varieties are complex inter-specific hybrids involving *S. officinarum and S. spontanium*

The sterility of distant hybrids may be caused by **cytogenetic, genetic or cytoplasmic** factors.

Cytogenetic Basis of sterility: Most of the inter-specific hybrids show reduced chromosome pairing and in extreme cases all the chromosomes may be present as univalents. The distribution of chromosome in such cases is irregular, and it leads to the formation of unbalanced gametes resulting in partial to complete sterility. For instance, inter specific crosses show rings and chains at metaphase-I (indicating translocations), bridges and fragments at anaphase-I (indicating inversions), loops at pachytene (indicating duplications or deletions). These cytological aberrations reduce fertility and the only solution to this is doubling the chromosome number i.e. by producing amphidiploids from them.

Genetic Basis of sterility: Chromosome pairing in some inter-specific hybrid is regular, but they show variable sterility which is due to genes. For example, F_1 hybrid between foxtail millet, *setariaitalica* and its wild relative *S. viridiss* howed normal pairing and regular formation of bivalents but pollen and ovule sterilities were 70 and 50% respectively.

Cytoplasmic Basis of sterility: In some inter-specific hybrids of *Epilobium and Oenothera*, sterility is produced by the cytoplasm (cytoplasmic male sterility factors). In such cases, the reciprocal crosses produce fertile hybrids.

Limitations of Distant Hybridization

1. Incompatible Crosses
2. F_1 Sterility
3. Problems in Creating New species
4. Lack of Homeology between Chromosomes of the Parental Species
5. Undesirable Linkages

6. Problems in the Transfer of Recessive Oligogenes and Quantitative Taits
7. Lack of Flowering in F_1
8. Problems in using improved varieties in Distant Hybridization
9. Dormancy

Achievements

1. Mainly in Wheat, Tobacco, Cotton
2. Parbhani Kranthi of Ladyfinger: Derived from *A. esculentuscv* Pusa Sawani x *A. Manihot* which is resistant to yellow mosaic vein virus, yield-Kharif: 110-120 q/ha, Summer: 85-90 q/ha.

Molecular Markers in Plant Breeding

Plant breeding where use of molecular markers is done in conjunction with linkage map and genomics to select plant with desirable traits on the basis of genetic assays.

Why molecular markers are preferred in Plant breeding?

a. Plant Cultivar identification: for authentication of cultivars and to establish a cultivar for patenting

b. Early generation testing: unlike phenotypic markers, don't have to wait for adulthood.

c. Rapid introgression of simply inherited traits: Avoids linkage drags and innumerable backcrosses to be made when the source of desirable genes is from a wild species.

d. Better characterization of breeding material for using them in breeding system: to help develop linkage map and understand the markers that co-segregate with QTLs to facilitate breeding of polygenic traits

e. Increasing life span of a cultivar: (Gene pyramiding) that is transferring multiple disease resistance genes and genes for yield in a sequential manner into one elite cultivar through transgenic approaches so that the CV remains superior and ahead of its time w.r.t the pest pressures and still with not much change in the genetic background.

Marker System Selection

Each marker system has advantages and disadvantages, so each system has to be evaluated prior to use. Selection of a DNA marker system for plant breeding depends on

1. Objectives of the research project
2. Population structure of the organism
3. Its genetic diversity (range of polymorphism)
4. Prokaryotic or eukaryotic system
5. Marker system availability and feasibility to use it effectively
6. Economic importance of the species
7. Time required for analysis and cost per unit information.

Next generation sequencing (NGS)

- High through put (Speed of sequencing very high, 20 million bases read in 6 hours).
- Useful for genome sequencing as well for metagenomics
- Fully automated, highly sophisticated
- Most important of the NGS methods are Sequencing DNA by microarray based hybridization (gene chips, phylochips), Massively parallel signature sequences (for cDNAs) and Pyrrosequencing. Pyrrosequencing is named after the use of inorganic pyrophosphate molecule released when DNa polymerase incorporates a free dNTP into a growing chain of DNA.
- In one pyrrosequencing machine there are 2 lakh microscopic wells, in them DNA is denatured and attached overa solid microscopic bead, along with primers, Taq Polymerase, Buffer with bivalent cations. BUT NO dNTPs ARE ADDED.
- This is one pyrrosequencing reaction with one bead/one well and thus a pyrrosequencer is capable of running 2 lakh different pyrrosequecing reactions with different ssDNA template. Although read per well is small (300-450bp only) but because of high magnitude of reactions going simultaneously.

Reactions of Pyrrosequencing

1. First of all, pyrrosequencing is of two types: solid phase and liquid phase. While in Solid phase, washing becomes imminent to proceed further because of accumulation of waste compounds making it difficult for the laser to catch the signal, this is not so in Liquid phase where there is no solid support so no washing is required and reaction can proceed for longer reads.
2. Through a microinjection one kind of dNTP's are added in working concentration inside the well. If that dNTP (say dATP) is what is required by the Taq polymerase to initae the reaction further, dATP will be incorporated once or twice or even more number of times depending on the template sequence (Case I). If dATP is not required by taq to proceed there will be no incorporation so no reaction (Case II).
3. In Case I, when the dATP is incorporated, there will be release of one pyrophosphate molecule per incorporation. This molecule of pyrophosphate will be used by enzyme **ATP sulfurylase** to make ATP which will in turn in the presence of Oxygen and substrate Luciferin and enzyme **luciferase** will lead to breakdown of ATP into AMP with release of bond energy which will used by the enzyme luciferase to emit light. The light signal is received by the LASER and the laser will inform the computer for a positive reaction as well for the number of dATPs incorporated as the light emitted by enzyme cascade is directly proportional to the amount of PPi (pyrrophosphate) released, it is easy to detect 5-6 base incorporation simulataneously.
4. In case II, no incorporation means no enzyme cascade and so no reaction. In this case, enzyme **Apyrase** or **exonuclease** or **alkaline phosphatase** (any one, not all!!) will be used to render all the dATPs present in the well useless for any further incorporation.

5. Be it case I or II, after a while before beginning a fresh injection into the well of dNTPs, all the already present dNTPS will be rendered useless as mentioned in point 4 above.
6. After this, there will fresh addition of a new kind of dNTP say dTTP and then again we will have case I or II depending on the template sequence.
7. This will go on until a point will come when because of accumulation of too many unused dNTPs, it will become difficult to make the light emitted in a positive reaction reaching laser unhindered thus making false reading or no reading when there is a signal.
8. At this point the reaction is terminated and depending on whether it is solid phase or a liquid phase washing may or may not be required to done as mentioned in point 1 above.

9.